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INTRACRANIAL CHORDOBLASTOMA *

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In 1846 R. Virchow noted on the surface of the clivus Blumenbachii a small slimy excrescence attached to a defect in the bone (Fig. 1). He spoke of it at the Würtzburger physikalisch-medizinischen Gesellschaft on February 24, 1856, and in 1857 gave it the name of *ecchondrosis physaliphora*. In the meantime the same structure had been described by Luschka (1856), but its true nature was first pointed out by Müller in 1858.

Müller showed from the study of human material that the ecchondrosis physaliphora was a persistent remnant of the notochord, but his work was not accepted as final and the nature of the malformation was disputed until the publication by Ribbert (1895) of his experiments on animals which proved conclusively the notochordal origin. In 1894 Ribbert suggested the name *chordoma* which has since been employed.

It was soon found that similar formations might develop all along the spinal canal, particularly at the sacral extremity. The records of the cases described have been collected in the reviews of Coenen (1925), of Corsy and Surmont (1927), and of Stewart and Morin (1926). The ecchondrosis physaliphora of the clivus Blumenbachii was soon discovered to be a fairly frequent malformation, being found in 2 per cent of necropsies by Ribbert and in 1.5 per cent by Stewart and Morin. Interest in it soon declined, although occasional reports occur in the recent journals (*cf.* Ajello, 1927), but was revived by the publication of cases in which it had reached a size suffi-

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cient to cause symptoms of intracranial tumor. The first record of this kind is that of Klebs (1864), in which is contained the account of a middle-aged man, said to have died of tetany. At necropsy was found a cloudy-white tumor attached to the clivus and measuring 1.5 by 2 by 1.8 cm. in diameter. Klebs was not sure that the tumor had caused the symptoms from which his patient suffered but undoubted instances have since been published to the number of about thirty-two, namely; the cases of Grahl (1903), Seiffer (1905), Fischer and Steiner (1907), Linck (1909), Frenkel and Bassal (1910), Jelliffe and Larkin (1911), Eitel (1911), Spiess (1911), Wegelin (1911), Hässner (1912), Kotzareff (1918), Argaud (1918–19), Daland (1919), Fabricius-Møller (1919), Hellmann (1921), Swars (1921), Lemke (1922), Mathias (1923), Hirsch (1923), Burrow and Stewart (1923), Arauz and Podestà (1923), André-Thomas and Jumentié (1923), Grossmann (1923), Filippini (1924), Eckel and Jacobs (1925), Citelli (1927), Castex and Camauër (1927), Camauër, Llambias and Mortola (1927), Sedláčkoviá-Šikl (1927), Argaud and Clermont (1928) and of Loebell (1928).

Details concerning most of the above tumors are readily available in the reviews of Stewart (1922) and of Stewart and Morin (1926). We may abstract the others briefly as follows:

Hirsch (1923). Mentioned in discussion of Grossman's case that he had a similar nasopharyngeal tumor, on which he operated by splitting the palate. It recurred a year later. No other details given.

Arauz and Podestà (1923). Female, 17. For twelve years difficulty of respiration and nasal speech. Tumor in left nasopharynx. X-ray showed lesion of axis. Paralysis of left external rectus. Vomiting but no headache. Developed slight hemiparesis. Biopsy; death fifteen days later. Illustrations show typical chordoma.

Filippini (1924). Female, 36. For seven years nasal voice. For three years difficulty of swallowing. Lately failing vision in right eye and finally disturbance of equilibrium. Right eye blind. Nystagmus on looking to right and left. Dysphagia. Paresis of right facial and of soft palate. Necropsy disclosed miliary tuberculosis. Tumor size of mandarin orange on clivus.

Citelli (1927). Male, 18. For one year difficulty in breathing. Bleeding from nose. Tumor filling nasopharynx. Biopsy; abundant hemorrhage. Partial removal. Recurred two years later.

Castex and Camauër (1927). Female, 20. Sleepiness and rapid oscillation of right eye. Later headache and vomiting. Dysarthria. Neuralgia in both jaws. Dysphagia. Paralysis of tongue. Right ptosis. Dilatation of right pupil. Paralysis right vocal cord; also right trapezius. Paresis both sixth nerves. Exaggeration tendon reflexes on left side. Died of pneumonia. Translucent tumor extending from optic chiasm to foramen magnum.

CAMAUËR, LLAMBIAS, AND MORTOLA (1927). Male, age not given. Transitory attack of loss of consciousness with weakness of left foot and double vision. Paresis right twelfth nerve. Left hemiparesis. No choking of optic discs. Died of pneumonia after a second attack. Small gelatinous tumor on clivus.

SEDLÁČKOVIÁ-ŠIKL (1927). Mentioned by Podlaha and Pavlicka. Details not available.

ARGAUD AND CLERMONT (1928). Female, 48. Small tumor of nasopharynx removed. Recurred several months later. Removed by trans-sinuso-facial route. Examination showed tumor to be chordoma. Said to have had "no ocular troubles."

LOEBELL (1928). Male, 62. Difficulty in breathing through left nares. Feeling of pressure in left ear, dizziness and faintness. Mass in left nasopharynx. Left third, sixth, and eighth palsy. X-ray showed sella turcica destroyed and erosion of petrous bone. Operated through antrum. Treated with radium. Recurred after one and a half years. Paralysis of both 3rd, 5th, 6th, 9th, and 10th nerves. Death. No necropsy.

To these records we wish to add two others, believing that in the case of so rare a tumor the publication of isolated instances in as much detail as possible is not only justifiable but necessary if a satisfactory general view of their structure, clinical course and treatment is to be obtained.

CASE REPORTS

Our first experience with these tumors occurred in 1928 when in examining the specimen from a supposed case of pituitary tumor we found the typical physaliferous cells. The record follows:

Case I. Intracranial Chordoblastoma with Symptoms of a Suprasellar Tumor. Transfrontal Exploration with Removal of what was taken for a Suprasellar Adenoma. Recovery.

On Jan. 4, 1928, Dr. A. M. Goldman of New York referred to the Peter Bent Brigham Hospital (Surg. No. 30402) a married Jewess, aged 34, complaining of headache and failing vision.

The family history was irrelevant. She began to menstruate at 14 years of age; was married at 20, and became pregnant but aborted after two months. A unilateral ovarectomy was performed. Since then her catamenia had been regular and normal. For about eighteen years she had recurrent headaches of gradually increasing frequency consisting of a dull ache above the inner angle of the left eye. During the past year she had gained about 20 pounds in weight. For the past three months her vision had been rapidly failing. Her physician stated that she had recently become somewhat childish and irritable and that her memory had been poor.

Examination on admission showed an alert and coöperative woman. There was found a bilateral primary optic atrophy (visual acuity 20/100 on either side), bitemporal hemianopsia, slight obesity (wt., 70 Kg.; ht., 1.58m), blood pressure slightly low (108/68), a slightly subnormal basal metabolic rate (−12), normal skin and distribution of hair, normal sella turcica, and normal urinary

output. There were no signs of involvement of the cranial nerves (apart from the optic), nor of the pyramidal nor cerebellar tracts.

The clinical diagnosis of suprasellar tumor of undetermined nature, possibly an hypophysial-duct tumor was made. On Jan. 14, 1928, Dr. Cushing made a transfrontal exposure with some difficulty due to a densely adherent dura mater. The optic chiasm was found pushed upward and forward by a smooth-surfaced, reddish white tumor suggesting a cyst. No fluid was obtained when the tumor was punctured with a needle. An incision was then made through the capsule and with a blunt spoon the contents were removed. There was very little bleeding from the raw surface.

The patient was restless and irritable, emotionally unstable and very talkative for several days after the operation. Her temperature rose to 103° F. The recovery was otherwise uneventful and she was discharged on Jan. 30, 1928. The visual acuity remained about the same as at admission, but there had been a slight expansion of the visual fields. She reported for examination on April 18, 1929, at which time there had been no appreciable change in her visual fields which still remained defective bitemporally; her acuity of vision in the left eye had considerably improved.

The tissue removed at operation was fixed in Zenker's fluid and in 10 per cent formalin. It was embedded in paraffin and sections were stained with hematoxylin and eosin, with hematoxylin-mucicarmine-metanil yellow, and with aniline blue-orange G.

Microscopic Description: The microscopic appearance is quite homogeneous. The tissue consists of cords and masses of epithelial cells having very indistinct intercellular boundaries (Fig. 2). The cytoplasm is dense and coarsely granular, and contains numerous large vacuoles. The nuclei vary greatly in size and shape, many being pyknotic. A few suggestive nuclear figures are seen but none which can unquestionably be identified as a mitosis. Between the cords of cells is a finely granular or stringy material which stains brightly red with mucicarmine; the cytoplasm of the cells by this method appears yellow.

Comment: It is evident that we have just described the typical vacuolated or physaliferous cells of a chordoma, arranged in cords and separated by a mucinoid substance. The tumor had not the usual semitranslucent gelatinous external appearance usually described but was soft and quite avascular and so bled very little when it was dug out.

The patient had symptoms of a suprasellar tumor, but few if any evidences of secondary hypophysial involvement. A case very similar, except for an erosion of the sella turcica visible in the roentgenogram, was described by Spiess. His patient was operated on by

the nasal route and the same type of tissue was found. In examining the X-rays of our patient after operation, Dr. Sosman remarked that "the posterior clinoids meet the basisphenoid at an acute angle and the basisphenoid appears defective about 1 cm. below this junction. However, it is quite difficult to locate a defect when it is at right angles to the plane of vision."

After identifying the tumor in this case as a chordoma, we searched through Dr. Cushing's pathological collection in the hope of finding other examples that had possibly been overlooked. The only case we were able surely to identify was one reported by Dr. Cushing in his monograph on The Pituitary Body and Its Disorders (Case XVII) as a teratoma. We believe it to be another chordoma and consequently reproduce the essentials of the record here.

Case II. Large Interpeduncular Tumor. Two Transsphenoidal Operations. Death. Necropsy. Chordoblastoma Originally Reported as a Teratoma.

On Oct. 5, 1909, there was admitted to the Johns Hopkins Hospital (Surg. No. 24773), J. McN., age 35, complaining of headache and loss of vision.

Past and family history were irrelevant. He had been married for seven years and had three children.

In 1903, for a period of six months, he suffered severely from frontal headaches and vomiting. During this time his vision failed, first in the left eye and later also in the right. There was also ptosis of the right eyelid and diplopia. The headaches became less severe but vision was progressively lost. In the last few months he lost completely his libido sexualis and gained 27 pounds in weight.

On admission he weighed 214 pounds and was 5 ft. 6 in. in height. There was almost complete blindness, only a small central field remaining in the left eye. The acuity was only 5/80. There was also a complete anosmia and almost complete external and internal ophthalmoplegia, there being only some slight movement of the left external rectus muscle. There seemed to be some slight choking superimposed upon a bilateral atrophy of the optic nerves. The patient complained of pain in the left trigeminal area but no objective evidence of involvement of this nerve was found. He was emotional, loquacious, irritable and forgetful. There was no polyuria. In the vault of the pharyx was a protrusion suggesting a vascular tumor. The X-rays showed that the dorsum and floor of the sella turcica were absent, but the anterior clinoid processes were present.

On Oct. 12, 1909, Dr. Cushing made a preliminary tracheotomy and then deflected the nose downward according to Eiselsberg's method and approached the tumor through the ethmoidal cells. The sphenoidal cells were obliterated but the bulging capsule of the tumor was recognized and some large fragments of the friable growth were removed. The wound was closed without drainage and healed promptly.

There was a slight polyuria for several days and his vision improved slightly. He was discharged Nov. 1, 1909. His headaches returned after two months and he soon regained the weight he had lost. He was readmitted April 18, 1910 and a more radical attempt was made on April 28 to remove the tumor. There was

considerable bleeding, necessitating packing of the cavity. The patient pulled the drains from his nostrils and died of hemorrhage during the night.

A complete necropsy was made. The general examination was not remarkable. The brain was removed after fixation *in situ* with the sphenoidal bone attached. There was a large friable mass of tumor about 5 cm. in diameter lying between the infundibulum and pons and pressing the peduncles forcibly upward (Fig. 3), compressing and elongating the 3rd, 4th, and 6th cranial nerves. The left trigeminal nerve was also compressed. The hypophysis was flattened against the anterior surface of the growth.

The tumor was preserved in 10 per cent formalin. Blocks were embedded in paraffin and sections stained with hematoxylin and eosin, and with hematoxylin mucicarmine-metanil yellow; others were impregnated by Perdrau's method.

Microscopic Description: The tumor consists essentially of large vacuolated cells arranged in cords and masses, with a varying amount of intercellular material (Figs. 4 and 5).

The cells are usually vacuolated in such a way as to leave a small amount of granular cytoplasm around the nucleus, but in many the vacuole lies excentrically. The nuclei vary enormously in size and shape; some are vacuolated. No mitoses are found.

When stained with mucicarmine and metanil yellow the cytoplasm appears yellow but the intercellular substance is bright red. The latter is usually amorphous or granular but in a few places is denser and homogeneous, having the appearance of cartilage. In these areas it has a reddish violet color in the Perdrau preparations; elsewhere it is not impregnated. In the cartilaginous regions occur granular deposits of calcium (Fig. 6).

A few thin-walled blood vessels are present, surrounded by cuffs of lymphocytes.

Comment: From the microscopic description and position of the tumor there can be no doubt of its nature. In Dr. Cushing's monograph the tumor is said to consist of "embryonic cartilage containing a few bone-cells with myxomatous and connective tissue." It was interpreted at the time by Dr. Welch as a "mixed tumor from a congenital anlage." According to our own examination the tumor consists of unmistakable notochordal tissue with a great amount of mucoid intercellular stroma in which scattered islands of cartilage are differentiating. The only bone we found was in the inferior and outer parts of the growth and might have been a fragment of invaded cranium. There were, however, scattered granular deposits of calcium such as were found by Hässner (1912).

Cartilage normally forms from the notochord during regeneration (Barfurth) and during embryonic development in lower vertebrates, perhaps also in mammals and man (Salvi, 1899). It should not be surprising, therefore, to find cartilage differentiating in chordomas.

Discussion

Gross Appearance: These tumors are almost invariably described as having a grossly nodular but smooth surface, of a milky-white color. The cut surface is semitranslucent resembling that of cartilage, but is of softer, often jelly-like, consistency. There are cysts filled with a slimy material resembling mucus. Other tumors are described as reddish or brownish, probably due to interstitial hemorrhage, although they are usually poorly vascularized and bleed only slightly when incised.

These growths arise in the base of the sphenoidal bone and may project either upward into the intracranial cavity from the clivus Blumenbachii or downward into the nasopharynx through the craniopharyngeal foramen. In several cases there was both an intracranial and an intrapharyngeal extension (Wegelin, Spiess, Mathias, Linck, Hellmann). There are a few cases recorded in which the tumor seems to have been confined to the nasopharynx (Argaud and Clermont, Grossmann, Fabricius-Møller), but in two of these there is no record of a neurological examination and in no instance has the absence of intracranial extension been proved by necropsy.

The tumor may extend into the sphenoidal sinuses, the orbits of the eyes or even into the neck (Daland, 1919) and regional veins (Wegelin 1912).

Microscopic Structure: The microscopic appearance of these tumors is remarkably uniform. The most characteristic feature is of course the vacuolated or physaliferous cells. It has been abundantly proved (Franco 1922, Grossmann 1923, Mathias 1921, Podlaha and Pavlica 1928) that the vacuoles are filled with glycogen. Since its demonstration demands fixation of the tissue in absolute alcohol we were unable to find it in our material. But we were able to show the presence of intercellular mucin, very abundant in Case I, less so in Case II. Sometimes the cells are widely separated by the mucin, causing a superficial resemblance to a chondroma; in others they are so closely packed that the tumor resembles a small-celled

carcinoma (Hellmann 1921). The cells are usually arranged in groups or cords.

The nature of the nuclear vacuolization, which was frequently seen in the tumor of our Case II, is still disputed. These vacuoles are said also to contain glycogen (Stewart, 1922).

Diagnosis: The microscopic diagnosis is fairly easy, but the clinical diagnosis presents difficulties. Kotzareff has said that the diagnosis during life is impossible. This is obviously not true of those tumors with a nasopharyngeal extension. Several such cases have been diagnosed by biopsy (Mathias, Loebell, Linck, Hirsch, Hellmann, Grossmann, Fabricius-Møller, Argaud and Clermont).

But the diagnosis of purely intracranial chordomas is certainly not easy. The symptoms, as one might suppose, consist essentially of palsies of the cranial nerves, from the third to twelfth pairs. In such cases of multiple palsies the possibility of a chordoma must be remembered. By extension forward to the optic chiasm a bitemporal hemianopsia may result (Spiess, our Case I); by distortion of the pons and peduncles pyramidal symptoms arise; by extension backward cerebellar symptoms are produced, and by increasing size symptoms of intracranial tension appear. However, although headache is common, choking of the optic discs is relatively rare and late in development. It is mentioned in six of the thirty-five cases; in two of them it was very slight. A more frequent complaint is pain on movement of the neck, doubtless due to extension of the tumor into the foramen magnum.

In a few cases (Spiess, Loebell, Kotzareff) destruction of the base of the skull has been demonstrated by the X-rays. It is possible that with better technique this destruction could be more often seen. In cases of basal tumor we suggest that a stereoscopic examination be made with the plates under the patient's chin and the rays coming from the parietal region. In this way the clivus Blumenbachii can be clearly outlined and small defects readily detected.

The principal tumors to be differentiated would seem to be the carcinoma of nasopharyngeal origin which has invaded the intracranial cavity, the hypophysial carcinoma, the meningioma of the cavum Mecklii, the neurinoma of the acoustic nerve, the tumors of the hypophysial duct, and perhaps a rare pearly tumor. Of these the acoustic tumors and the tumors of the hypophysial duct have such a definite chronology and symptomatology that the confusion need

rarely arise; the pearly tumors do not erode the bone; and the palsies caused by meningiomas of the cavum Mecklii are unilateral. But the carcinomas of nasopharyngeal or hypophysial origin are more difficult to differentiate. We know little of the latter tumors. It is to be hoped that the forthcoming study of Oljenick will aid in their recognition. Nasopharyngeal carcinomas can of course often be diagnosed by examination of a specimen removed from the original tumor in the nasopharynx.

In conclusion we would say that in any case of multiple palsies of the cranial nerves the possibility of a chordoma should be kept in mind; an examination of the nasopharynx or a proper roentgenological study might aid in establishing the correct pathological diagnosis.

Treatment: Study of specimens at necropsy gives little hope that these tumors can ever be completely removed. Vincent is said to have removed the tumor from the clivus in Argaud's first case. The condition of the patient afterward is not mentioned; one may well imagine it. Attempts to reach the tumor by transfrontal (Cushing), temporal (Kotzareff), suboccipital (André-Thomas and Jumentié), transnasal (Spiess) or transmaxillary (Loebell) operations have been equally unsuccessful. The nasopharyngeal extensions have been removed and treated by radium (Loebell) or X-ray (Daland) but have invariably recurred after a short interval.

BIBLIOGRAPHY

Ajello, L. Contributo casistico allo studio del cordoma del clivus di Blumenbach. *Cultura med. mod.*, 1927, vi, 39–48.

Arauz, S. L., and Podestà, R. Cordomas malignas del cavum. *Prensa méd. argent.*, 1923–24, x, 461–471.

Argaud, R. Chordome malin. *Arch. de méd. expér. et d'anat. path.*, 1918–19, xxviii, 364–374.

Argaud, R., and Clermont, D. Chordome primitif malin de la poche de Luschka. *Ann. d'anat. path.*, 1928, v, 145–152.

Bailey, P. Cruveilhier's "tumeurs perlées." *Surg. Gynec. Obst.*, 1920, xxxi, 390–396 (Case 2).

Burrow, J. le F., and Stewart, M. J. Malignant spheno-occipital chordoma. *J. Neurol. & Psychopath.*, 1923, iv, 205–217.

Camauër, A. F., Llambias, C. A., and Mortola, G. A. Un caso de Cordoma. *Rev. oto-neuro-oftal.*, 1927, i, 370–374.

Castex, M. R., and Camauër, A. F. Estudio anátomo-clínico de un caso de cordoma de la base ósea del cráneo. *Prensa méd. argent.*, 1927, xiv, 10–29.

Citelli, S. Su un caso di cordoma maligno della volta faringea. *Valsalva.*, 1927, iii, 197–202. *Cf.* also *Zentrlbl. f. Hals-, Nasen-u. Ohrenh.*, 1927, ix, 46.

Coenen, H. Das Chordom. *Beitr. z. klin. Chir.*, 1925, cxxxiii, 1–77.

Corsy, F., and Surmont, J. Sur l'histogenèse et l'évolution des tumeurs de la notochorde. *Bull. de l'Assoc. franç. p. l'étude du cancer.*, 1927, xvi, 316–376.

Cushing, H. The Pituitary Body and its Disorders. (Case 17.) Lippincott, Philadelphia, 1912.

Daland, E. M. Chordoma. *Boston M. & S. J.*, 1919, clxxx, 571–576.

Eckel, J. L., and Jacobs, W. F. Malignant spheno-occipital chordoma. *J. Nerv. & Ment. Dis.*, 1925, lxi, 471–486.

Eitel, W. Ein Beitrag zur Kasuistik der Chordome. Inaug. Diss. Heidelberg. 1911.

Fabricius-Møller, J. Et Tilfaelde af Chordoma malignum med Indvaekst i Pharynx. *Hospitalstid.*, 1919, lxii, 849–857.

Filippini, G. Sopra un caso di cordoma a tipo maligno. *Riv. dell'osp. magg. di Milano*, 1924, xii, 307–312. *Cf.* also *Centralbl. f. allg. Pathol. u. path. Anat.*, 1924–25, xxxv, 469.

Fischer, B., and Steiner. Über ein malignes Chordom der Schädel-Rückgratshöhle. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1907, xl, 109–119. *Cf.* also Steiner: *Allg. Ztschr. f. Psychiat.*, 1905, lxii, 243.

Franco, E. E. Considerazioni intorno ad un cordoma. *Arch. per le sc. med.*, 1922, xlv, 310–327.

Frenkel, H., and Bassal, L. Sur un cas de chordome malin sphéno-occipital *Arch. de méd. expér. et d'anat. path.*, 1910, xxii, 703–728.

Grahl, O. Eine Ecchondrosis physalifora spheno-occipitalis ungewöhlichen Umfangs mit interessanten klinischen Folgen. Inaug. Diss. Göttingen, 1903.

Grossmann, B. Fall von malignen Chordom des Nasenrachenraumes. *Monatschr. f. Ohrenh.*, 1923, lvii, 610–611 and 1051.

Hässner, Dr. Ueber Chordome unter gleichzeitiger Mitteilung eines Falles von seltener Grösse. *Virchows Arch. f. path. Anat.*, 1912: ccx, 385–406. *Cf.* also Schwalbe; *Zentralbl. f. Chir.*, 1912, xxxix, 1326.

Hellmann, K. Ein malignes Chordom des Nasenrachenraumes. *Verhandl. d. Gesellsch. deutsch. Hals-, Nasen- u. Ohrenärzte.*, 1921, 111–117.

Hirsch, Dr. Diskussion zu Grossmann. *Monatschr. f. Ohrenh.*, 1923, lvii, 1052.

Jelliffe, S. E., and Larkin, J. H. Ueber ein malignes Chordom mit Symptomen von seiten des Gehirns und Rückenmarks. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1911, v, 590–604. *Cf.* also Jelliffe, *J. Nerv. & Ment. Dis.*, 1910: xxxvii, 442; and Larkin, *Tr. New York Path. Soc.*, April 1910.

Klebs, E. Ein Fall von Ecchondrosis spheno-occipitalis amylacea. *Virchows Arch. f. path. Anat.* 1864, xxxi, 396–399.

Kotzareff, A. Un cas de chordome. *Cor.-Bl. f. schweiz. Aerzte.*, 1918, xlviii, 1007–1014.

Lemke, R. Ein Fall von malignem Chordom der Schädelbasis. *Virchows Arch. f. path. Anat.*, 1922, ccxxxviii, 310–324.

Linck, A. Chordoma malignum, ein Beitrag zur Kenntnis der Geschwülste an der Schädelbasis. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1909, xlvi, 573–585.

Loebell, H. Zur Therapie der bösartigen Clivus- und Nasenrachenchordome. *Ztschr. f. Hals-, Nasen- u. Ohrenh.*, 1928, xxi, 337–348. *Cf.* also Siegmund; *Verhandl. d. deutsch. path. Gesellsch.*, 1923, xix, 201.

Luschka, H. Die Altersveränderungen der Zwischenwirbelknorpel. *Virchows Arch. f. path. Anat.*, 1856, ix, 311–328.

Mathias, Herr. Ein Beitrag zur Lehre vom malignen Chordom. *Verhandl. d. deutsch. path. Gesellsch.*, 1923, xix, 198–202. *Cf.* also Goercke; *Verhandl. d. Gesellsch. deutsch. Hals-, Nasen- u. Ohrenärzte*, 1921, 117.

Müller, H. Ueber das Vorkommen von Resten der Chorda dorsalis bei Menschen nach der Geburt und ueber ihr Verhältnis zu den Gallertgeschwülsten am Clivus. *Ztschr. f. rationelle Med.*, 1858, ii, 202–230.

Oljenick, Ign. Hypophysial carcinomas. (In press.)

Podlaha, J., and Pavlica, F. Das bösartige sacrococcygeale Chordom. *Virchows Arch. f. path. Anat.*, 1928, cclxvii, 363–378.

Ribbert, H. Ueber die Ecchondrosis physalifora spheno-occipitalis. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1894, v, 457–461.

Ribbert, H. Ueber die experimentelle Erzeugung einer Ecchondrosis physalifora. *Verhandl. d. Kong. f. inn. Med.*, 1895, xiii, 455–464.

Salvi, G. Sopra la sparizione del segmento vertebrale della chorda dorsale in rapporto con l'ossificazioni dei corpi delle vertebre. *Monitore zool. ital.*, 1899, x, no. 8.

Sedláčkoviá-Šikl. *Rev. v. Neurol. Psychiatr.* (Haskovec); March, 1927. Quoted from Podlaha and Pavlica (*loc. cit.*).

Seiffer, Herr. Ein Fall von seltener Geschwulstbildung der Schädelbasis. *Neurol. Centralbl.*, 1905, xxiv, 469.

Spiess, G. Tumor der Hypophysengegend auf endonasalem Wege erfolgreich operiert. *München. med. Wchnschr.*, 1911, ii, 2503. *Cf.* also Knoblauch; *Versamml. d. Gesellsch. deutsch. Nervenärzte* (Frankfurt-am-Main) Oct. 4, 1911.

Stewart, M. J., Malignant sacrococcygeal chordoma. *J. Path. & Bact.*, 1922, xxv, 40–63.

Stewart, M. J., and Morin, J. E. Chordoma; a review, with report of a new sacrococcygeal case. *J. Path. & Bact.*, 1926, xxix, 41–60.

Swars, Dr. Ueber einen neuen Fall von malignem Chordom. Inaug. Diss., Königsberg, 1921.

André-Thomas, and Jumentié. J. Chordome de la région sphéno-basilaire, *Rev. neurol.*, 1923, xxxix, i, 300–304.

Virchow, R. Untersuchungen ueber die Entwickelung des Schädelgrundes. Georg Reimer, Berlin, 1857, 51.

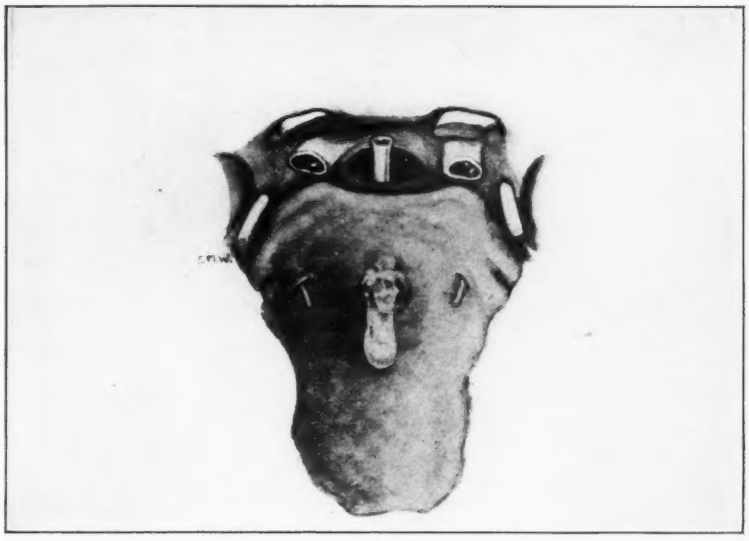
Wegelin, K. Ueber ein malignes Chordom des Clivus Blumenbachii. *Atti d. Cong. internaz. dei Patologi.* (Torino, 1911) 1912, i, 70. *Cf.* also *Centralbl. f. allg. Pathol. u. path. Anat.*, 1912, xxiii, 11.

DESCRIPTION OF PLATES

PLATE 88

FIG. 1. Ecchondrosis physaliphora (After Burrow and Stewart).

FIG. 2. Case I. Photomicrograph of tumor. Methylene blue-eosin. Note the cords of vacuolated cells separated by mucinoid material. × 300.



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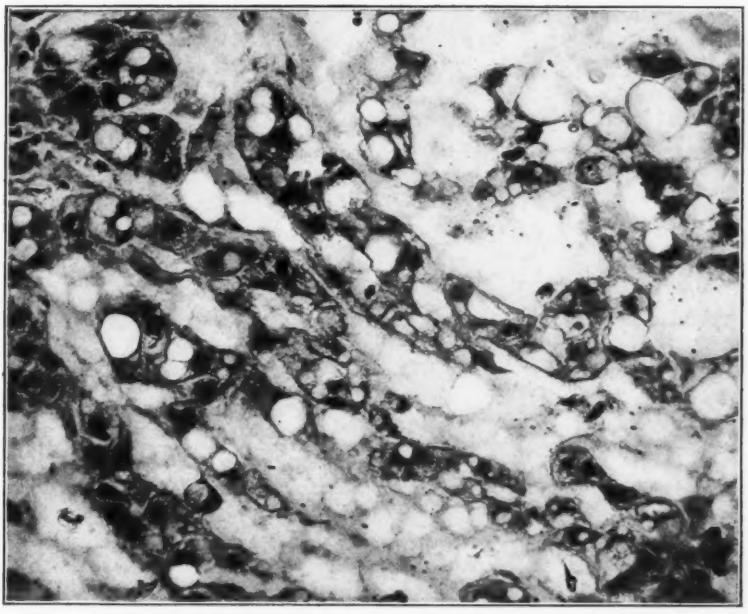


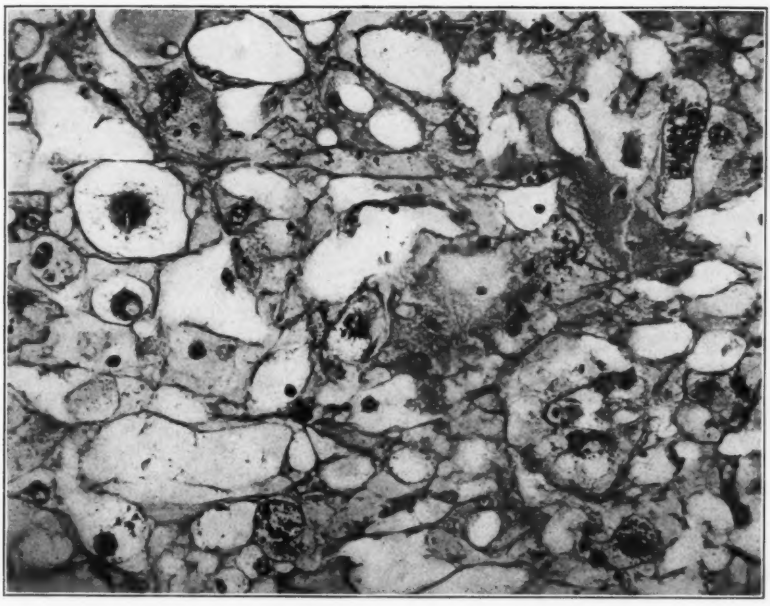
2

PLATE 89

FIG. 3. Case II. Median section of brain with tumor in place. Note its position between the infundibulum and pons.

FIG. 4. Case II. Photomicrograph of tumor. Iron-hematoxylin. Vacuoles are present both in the cytoplasm and nuclei of the cells. × 300.

3



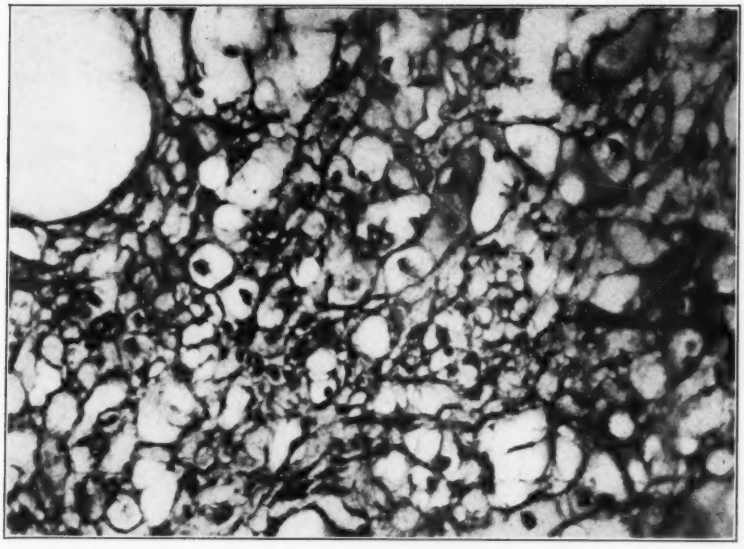
4

Bailey and Bagdasar Intracranial Chordoblastoma

PLATE 90

FIG. 5. Case II. Cords of vacuolated cells are seen. Mucicarmine-metanil yellow. × 325.

FIG. 6. Case II. Calcium salts being deposited in the mucoid stroma. Hematoxylin and eosin. × 300.



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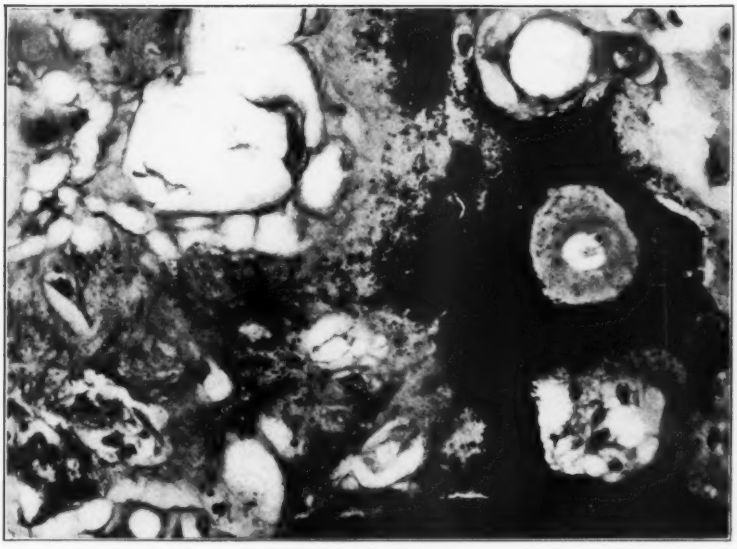

6

INTRAVITAL STAINING OF MICROGLIA WITH TRYPAN BLUE*

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It is commonly accepted that the intravital selection of the benzidine dyes from colloidal solution is characteristic of the cells of the so-called reticulo-endothelial system (Kiyono,[1] Aschoff[2]). Those to whom the name "reticulo-endothelial system" is objectionable will still admit the existence of a macrophage system with vital staining properties in the body whether these macrophages are labelled clasmatocytes, endothelial cells, Marchand's adventitial cells, or collectively as histiocytes. Excluding the central nervous system these macrophages have been extensively studied; the reader is referred to Aschoff's [3] paper and to Maximow [4] for reviews of the work and the relevant literature.

The macrophages of the central nervous system have as yet been imperfectly studied with the vital stain technique from the point of view of their relationship to, and possible origin from, glia. This is doubtless due, in part, to the recent development of the metallic impregnation methods of the Spanish school by which the different types of glia can be distinguished. Although vital staining has been applied to the brain, the procedure has not yet been combined with subsequent staining of sections by the silver carbonate methods of Hortega. Thus Macklin and Macklin [5] were able to produce a striking coloration of the tissues about cerebral puncture wounds in rats, using trypan blue intravitally, and they described the appearance of granules of dye in the macrophages in such areas. They thought that some of the neuroglia cells also contained blue granules, but without special staining methods they were unable to trace the relationship of their macrophages to the glia. More recently Kubie [6] applied Sabin's supravital technique to the same problem with the object of elucidating the relationship of microglia to the phagocytes of the brain. He demonstrated the presence of lymphocytes and clasmatocytes in the perivascular tissues of normal rabbits and guinea pigs by the supravital method, and intravitally in a rabbit

* Received for publication June 10, 1929.

that had received repeated small injections of trypan blue in the subarachnoid space. He could reach no conclusions concerning the part played by microglia in phagocytosis. His inability to do so may be attributed to his apparent omission of the silver impregnation methods for demonstrating microglia. Moreover, he does not appear to have made use of the microglial reaction round cerebral puncture wounds, although he produced such punctures in six of his twenty-two animals.

The vivid staining reaction obtained by Macklin and Macklin together with their suggestive observations on the deposition of trypan blue granules in the macrophages about puncture wounds, encouraged the view that a repetition of their procedure combined with specific staining of the microglia would throw some light on this relationship.

The microglial reaction around a sterile puncture wound of the cerebrum has been described and figured by Hortega [7] and by Hortega and Penfield.[8] Their observations have recently been repeated by Carmichael.[9] The striking accumulation of microglia cells in the periphery of the wound and the gradual transition between the normal resting form of the cell with its numerous branched processes and the rounded-up, phagocytic forms, known as compound granular corpuscles or *Gitterzellen*, are clearly demonstrated by these authors. The minute cytological changes involved in this transition have recently been described by Cone.[10]

Experimental

The rabbit was used for this work on account of the ease with which microglia can be demonstrated in this animal. The cerebral puncture was performed under ether anesthesia by tapping through the skull a three-inch nail the tip of which had been filed down to a fine tapering point. The nail was flamed before use but was allowed to cool down before being brought into contact with the tissues. The skin had been previously prepared by cutting the hair close and applying tincture of iodine. In no instance was there any evidence of bacterial infection.

A preliminary microscopic study of the reaction about the wound obtained in this manner showed that the optimum time for observing transitional forms of microglia lay between the second and fourth days after the operation. The reaction obtained at such a

stage is seen in Figs. 7 and 8.* It was therefore decided to observe the effects of intravital staining at this optimum period. Incidentally Macklin and Macklin found that the best intravital staining of the macrophages was also demonstrated within the same period, an observation that added point to the investigation.

Cerebral puncture was therefore repeated in conjunction with intravital staining. The trypan blue was administered by the intraperitoneal route following the directions given by Foot.[11] The sample of dye used was obtained from the National Aniline and Chemical Co. The dose used at first was 10 cc. of a 1 per cent solution of the dye in distilled water per kilo of body weight, but it was felt that the histological results obtained left room for improvement. The dose was then doubled without evidence of any toxic effect upon the animal, and there is little doubt that the dye could be pushed further if an even stronger reaction were desired. The first dose was given three hours before the brain puncture was performed and was then repeated at twenty-four hourly intervals. At the end of three or four days the animal was killed with chloroform, and the brain immediately transfused through the carotid artery with formalin ammonium bromide. Later the brain was removed and transferred in suitable blocks to a bottle of the same fixative. Sections were cut at 15 microns on the freezing microtome a few hours later, or on the following day, and were stained for microglia by Hortega's ammoniacal silver carbonate method (Hortega,[12] see also Penfield[13]). In using the method it was found advisable to leave the sections in the silver bath for less than thirty seconds, for a heavy impregnation is liable to obscure the granules of trypan blue, especially in the early transitional forms of microglia. Also prolonged toning in gold will change the blue of the dye to a purplish tint that is indistinguishable from the surrounding cytoplasm; for this reason the time in the gold bath was not longer than ten minutes.

Description of Results

The rabbits showed rapid absorption of the dye from the peritoneal cavity. In the course of two hours after the first injection the skin, conjunctivae and gums assumed a distinct blue tinge, and the

* These two figures are reproduced here with the kind permission of Dr. Penfield; they have already been published by Hortega and Penfield.[8]

color became more brilliant as time progressed. The brain wound was therefore produced at a time when the trypan blue had access to the site of injury. After twenty-four hours the rabbits excreted bright blue urine. There was no evidence that the dye exerted any toxic action upon the animal in these experiments.

On postmortem examination there was intense deep blue staining of the abdominal and thoracic viscera and of the soft tissues generally. Where the smaller dose of trypan blue had been employed the cerebral puncture wounds showed a faint blue halo only around the central clot. When the larger dose had been administered there was a bright blue halo, the macroscopic effect resembling that figured by Macklin and Macklin in their experiments on the rat. The rest of the cortex appeared normal. There was a brilliant blue staining of the choroid plexuses, and the leptomeninges showed many fine dots and streaks of blue, especially in the deeper sulci.

Microscopically there is a microglial reaction about the wound, of the type that has been described by Hortega and Penfield (Figs. 7 and 8). The track of the wound is occupied by recent blood clot in which a few *Gitterzellen* containing numerous granules of trypan blue are scattered. In the borders of the wound every type of transition can be traced between normal resting microglia and rounded *Gitterzellen*. On examination with high powers of the microscope discrete granules of trypan blue of variable size can be detected in the cytoplasm of all these transitional types (Figs. 2 to 6 inclusive), but, as might be expected, the blue is most abundant in the typical *Gitterzellen* and is relatively difficult to find in microglia cells that have undergone little change (Fig. 2). Almost all the trypan blue in the tissues about the wound can be accounted for by this intravital selection of the dye by microglia and its derivatives.

Granules of dye are also conspicuous in ameboid or spindle-shaped cells lying in the adventitia of the vessels in the neighborhood of the wound. These cells correspond without doubt to the clasmatocytes demonstrated supravitally by Kubie. They can be identified also as Marchand's adventitial cells. There are also granules of dye in round and spindle cells in the leptomeninges. Occasionally a few cells containing granules of trypan blue, which are not impregnated with silver when treated by Hortega's method for microglia, are found in the brain tissue adjacent to the wound. These cells are probably monocytes that have migrated from the blood stream.

They are relatively scarce in comparison with microglia cells in the early stages of reaction in these wounds and assume a more conspicuous part in the histological picture in the later stages, for example in wounds examined one week after the injury. Attempts to demonstrate granules of dye in the astrocytes by Hortega's silver carbonate method were unsuccessful.

In the rabbits used there was no evidence of that form of focal chronic encephalitis that is apt to appear spontaneously in laboratory animals of this species.

Discussion

The presence of numerous granules of trypan blue in the compound granular corpuscles, or *Gitterzellen*, and in their antecedent ameboid forms calls for little comment as an isolated observation. The origin of compound granular corpuscles, however, has been a subject of controversy. According to Alzheimer [14] these cells arise either from mesodermal tissues or from neuroglia. Metz and Spatz [15] consider that they are derived from microglia, but regard microglia as a form of neuroglia and hence of ectodermal origin. Nissl [16] traced them to a mesodermal source, and it is believed in many quarters at the present time, on the basis of Hortega's work, that microglia furnishes the greatest number of these phagocytic cells in pathological conditions of the brain, and that microglia is of mesodermal origin.

Whether all the *Gitterzellen* are derived from microglia or whether some of them may or may not arise more directly from other mesodermal sources (for example from the adventitial clasmatocytes) is outside the scope of this paper. The demonstration of granules of trypan blue in all transitional forms between typical microglia and typical *Gitterzellen* serves not only as additional evidence of their direct relationship, but also places microglia on an unequivocal footing as a part of the reticulo-endothelial system. It may be objected that microglia is precluded from this category because one cannot demonstrate granules of dye in the cytoplasm when the cell is in its resting form (Fig. 1). It is recognized, however (Maximow [4]), that the ability of macrophages in general to absorb the colloidal dyes is linked up with their phagocytic or ameboid activity. It is necessary, therefore, to employ a stimulus such as a simple aseptic puncture wound to activate these cells, and hence to demonstrate their true nature.

Conclusions

1. The intravital staining of microglia with trypan blue is demonstrated, using aseptic puncture wounds of the cerebrum as a stimulus to phagocytic activity.

2. It is claimed that such intravital staining identifies microglia with the rest of the reticulo-endothelial system of the body.

3. These conclusions offer further support to Hortega's contention that microglia is a mesodermal element.

4. There is no evidence of the formation of *Gitterzellen* from neuroglia or that neuroglia is able to take a vital stain.

My best thanks are due to Dr. Penfield and to Dr. Cone for much guidance and advice.

REFERENCES

1. Kiyono, K. Die vitale Karminspeicherung. Jena, 1914.

2. Aschoff, L. Lectures on Pathology. New York, 1924.

3. Aschoff, L. Das Reticulo-endotheliale System. *Ergebn. d. inn. Med. u. Kinderh.*, 1924, xxvi, 1.

4. Maximow, A. The Macrophages or Histiocytes. Special Cytology. E. V. Cowdry, ed., New York, 1928, i, 425.

5. Macklin, C. C., and Macklin, M. T. A study of brain repair in the rat by use of trypan blue, with special reference to the vital staining of the macrophages. *Arch. Neurol. & Psychiat.*, 1920, iii, 353.

6. Kubie, L. S. A study of the perivascular tissues of the central nervous system, with the supra-vital technique. *J. Exper. Med.*, 1927, xlvi, 615.

7. Río-Hortega, P. del. Poder fagocitario y movilidad de la microglia. *Bol. d. l. Soc. Esp. d. Biol.*, 1919, ix, 154.

8. Río-Hortega, P. del, and Penfield, W. Cerebral cicatrix; reaction of neuroglia and microglia to brain wounds. *Bull. Johns Hopkins Hosp.*, 1927, xli, 278.

9. Carmichael, E. A. Microglia: an experimental study in rabbits after intracerebral injection of blood. *J. Neurol. & Psychopath.*, 1929, ix, 209.

10. Cone, W. Acute pathologic changes in neuroglia and in microglia. *Arch. Neurol. & Psychiat.*, 1928, xx, 34.

11. Foot, N. C. Handbook of Microscopical Technique. C. E. McClung, ed., New York, 1929, 79.

12. Río-Hortega, P. del. El "tercer elemento" de los centros nerviosos. *Bol. d. l. Soc. Esp. d. Biol.*, 1919, ix, 69.

13. Penfield, W. Handbook of Microscopical Technique. C. E. McClung, ed., New York, 1929, 380.

14. Alzheimer, A. Histologische Studien zur Differenzialdiagnose der progressiven Paralyse. *Histol. u. histopath. Arb. Nissl-Alzheimer*, 1904, i, 18.

15. Metz, A., and Spatz, H. Die Hortegaschen Zellen (das sogenannte "dritte Element") und über ihre funktionelle Bedeutung. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1924, xxxix, 138.

16. Nissl, F. Zur Histopathologie der paralytischen Rindenerkrankung. *Histol. u. histopath. Arb. Nissl–Alzheimer*, 1904, i, 315.

DESCRIPTION OF PLATES

PLATE 91

Intravital staining of microglia in border of stab wound of cerebrum of rabbit four days after operation. Twenty cc. of 1 per cent trypan blue (5 doses) intraperitoneally.

FIG. 1. Normal resting microglia cell in hippocampus.

FIG. 2. Early transition. Scanty granules of trypan blue in cytoplasm.

FIG. 3. Two cells showing later stage with granules of trypan blue in swollen cytoplasm. The left-hand cell shows typical spinous branches of microglia at upper extremity.

FIG. 4. Later change toward ameboid form. Trypan blue granules more abundant and processes almost lost.

FIG. 5. Late transition stage; ameboid form of microglia.

FIG. 6. Typical compound granular corpuscle or *Gitterzell*.

Camera lucida drawings. × 900.

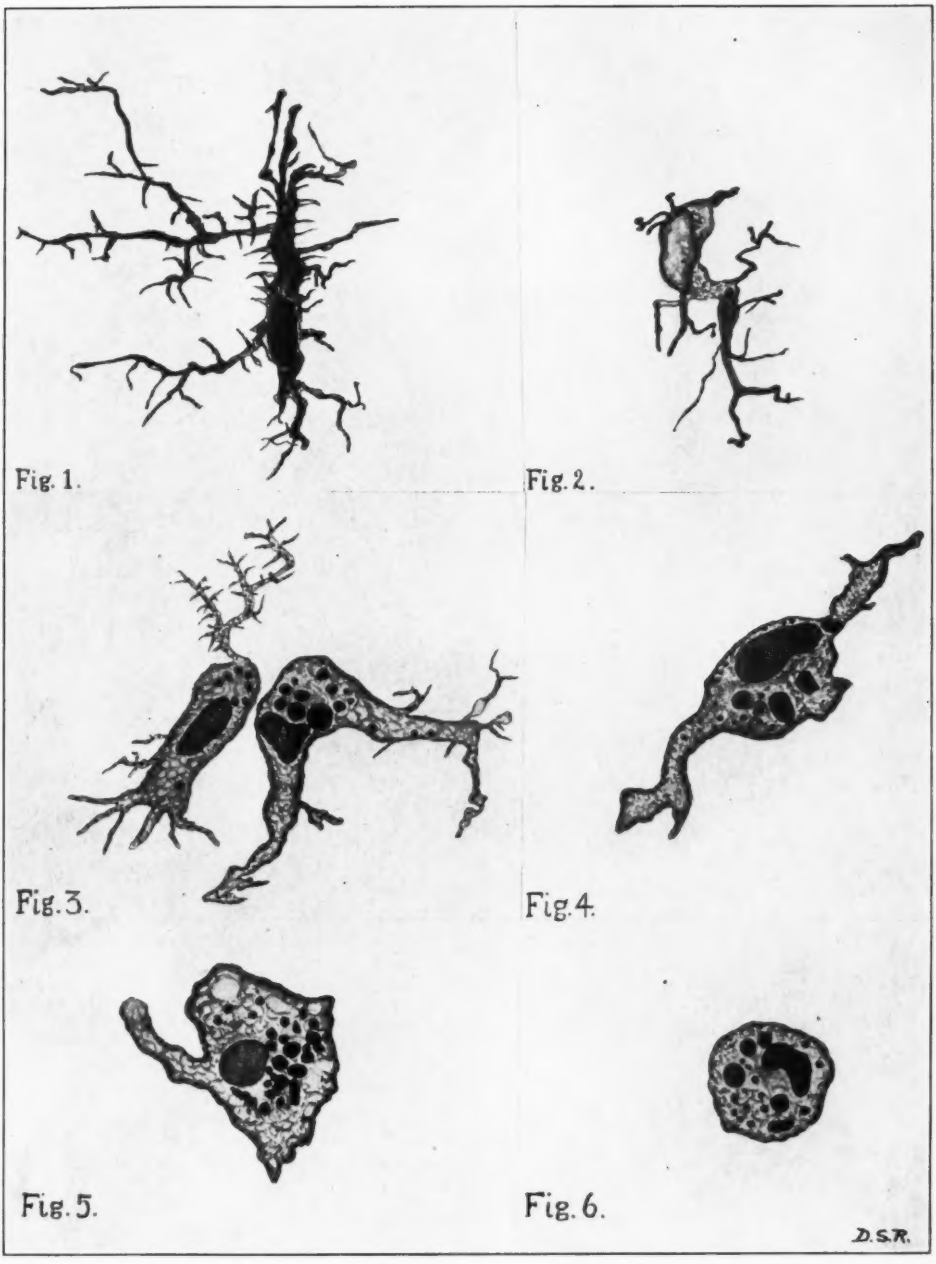

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

D.S.R.

PLATE 92

FIG. 7. Compound granular corpuscle forms of microglia at the border of a stab wound (T) made in the rabbit's brain two days previously. Silver carbonate microglia method.

FIG. 8. Same case as Fig. 7, higher power. Farther from the tract (T) there were seen transition stages between the branching form and this ameboid form of microglia. Silver carbonate microglia method.

(These photomicrographs are reproduced with the kind permission of Dr. Penfield.)

7

8

ABNORMALITY OF THE CEREBRUM AND LEPTOMENINGES SIMULATING AN INTRACRANIAL TUMOR *

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Localized gross abnormalities in the development of the brain are rarely met with in a neurosurgical clinic and may well be mistaken for brain tumors. Recently, the only large verified abnormality of the cerebrum observed in this clinic was considered as a tumor from its appearance. Had this lesion been exposed at operation it would undoubtedly have been considered as a tumor. Further, it is possible that if a piece of the tissue had been removed at operation the immediate diagnosis of an astrocytoma would have been made.

There are many interesting findings brought out in the study of the lesion to be described, but the purpose of this report is to present it from the standpoint of its clinical and gross resemblance to a tumor and to mention certain histological findings in the undeveloped meninges of interest in relation to the origin of the meninges.

Report of Case

P. B. B. H. Surg. No. 32960. Case of suspected brain tumor in an infant. Convulsions and asymmetrical enlargement of head developed soon after birth. Ventriculograms and examination pointed to cerebral tumor. No operation. Death from bronchopneumonia. Autopsy.

Clinical History: Arnold S., aged six months, was brought to the Children's Hospital of Boston on December 19, 1928, for observation. A history taken there by Dr. L. K. Diamond brought out the following facts. The infant was a second child of healthy parents. Their first child is living and well. This baby was born after a rather difficult labor, but at birth showed no evidence of injury. It was noted that there were six toes on each foot. During the first few days of life the child appeared to act normally in every respect. At the end of the first week, however, it was apparent to all that its head was becoming abnormally large. At two weeks the first convulsive attack occurred. This consisted of flexion of all four limbs, rolling up of the eyes and deep labored breathing. The attack lasted from three to four minutes. Similar attacks followed daily from then on.

Physical examination showed an apathetic, poorly developed infant with a large occipital bulge to the head. The head measured 52.5 cm., the chest 42.5

* Received for publication June 7, 1929.

cm., and the abdomen 40 cm. The height was 61 cm. The superficial vessels of the scalp were dilated, and the cranial sutures and fontanelles were open. Eyes showed internal strabismus, but no downward displacement of the eyeballs. Liver edge, spleen and left kidney were palpable. There was a slight lordosis with marked scoliosis. Six toes were seen on each foot. Knee and ankle jerks were hyperactive.

The cerebrospinal fluid was under increased pressure, sterile, contained no globulin and no abnormal cells. The blood and spinal Wassermann, general blood, stool and urine examinations were negative. The results of ventriculograms reported by Dr. E. C. Vogt were as follows:

"The right ventricle appears dilated and definitely larger than the left with a constricted area posteriorly. Repeated studies showed the same marked asymmetry of lateral ventricles with what is evidently a soft tissue mass depressing the right ventricle posteriorly from without."

The infant was considered a brain tumor suspect and transferred to the surgical service of this hospital December 31, 1928.

Examination here was essentially the same except that no epigastric or abdominal reflexes were obtained. It was felt that the deep reflexes of the legs were greater on the left. Beginning bronchopneumonia of both lower lobes was found.

The first impression of the case was in accord with a possible right cerebral tumor. Further studies were withheld, and all efforts directed to the bronchopneumonia, which, however, proved fatal in two days. Permission to examine the head only, was obtained.

Necropsy: The head was asymmetrically enlarged by a distinct bulge of the entire right side. The cranial sutures were widely separated. The thin cranial cap was removed with difficulty because of the adherent dura mater. When the dura was reflected there was apparent a large firm tumor-like mass in the right frontoparietal lobe (Fig. 1). *In situ*, this mass was of hard rubber consistency. The falx cerebri was pushed to the left. The brain weighed 980 gm.

The blood vessels over the base of the brain and cerebral hemispheres, elsewhere than the tumor mass, appeared normal grossly. Over the tumor area there were seen several large confluent venous channels lying in incomplete sulci (Fig. 1). There was also a decrease in the number of small superficial vessels over the same area as compared with the corresponding area of the normal appearing left side (Fig. 1).

The tumor-like mass replaced the superior half of the entire frontal lobe and extended back to the right paracentral region of the parietal lobe. Anteriorly the mass was sharply marked off from what remained of the frontal lobe. Laterally and posteriorly the lesion was less sharply demarcated, and, indeed, it was hard to tell where it ended in the gradual transition to the normal sulci and gyri. An-

teriorly the mass was like one huge gyrus with only psuedo-sulci, shallow umbilicated areas and local thickenings of the leptomeninges to interrupt its smoothness.

The leptomeninges over this area only were abnormal. They were firmly adherent to the cortex and were studded with multiple, irregular local translucent, gray-white thickenings of from 1 to 11 mm. in any diameter (Fig. 2). These areas were not related to blood vessels although occasionally blood vessels appeared superficially imbedded in them. They did not resemble cerebral herniae but suggested in the gross either local inflammatory reactions or masses of tumor cells in the subarachnoid spaces.

The remainder of the brain and a small portion of the cervical cord appeared normal externally and in comparison with several other brains of infants of the same age.

After fixation a series of coronal sections demonstrated the error in believing this lesion to be a tumor; for there was a gross abnormality corresponding with the lesion seen externally as well as an asymmetrical hydrocephalus involving the lateral and third ventricles. The many sections through the abnormal area showed it to be composed of a translucent white zone of 1 to 3 mm. in depth, beneath which there was a thickened gray matter and a decreased amount of white matter (Fig. 3). About the dilated right lateral ventricle the white matter was never more than 4 mm. in thickness. Both the gray and white matter appeared much firmer than the corresponding tissue in the left hemisphere.

The asymmetrical hydrocephalus involved the lateral and third ventricles. The capacity of the right lateral ventricle was about twice that of the left lateral ventricle. The foramina of Monro were dilated, equal, and measured 1 cm. in diameter. The aqueduct of Sylvius, the fourth ventricle and the basal cysternae appeared of normal capacity. The foramina of Magendie and Luschka were not demonstrated. The only abnormality of the ependyma consisted of a fenestrated dam-like thin membrane 1 cm. in height arising from the posterior portion of the floor of the anterior horn of the right lateral ventricle (Fig. 3). The choroid plexus, basal ganglia, cerebellum, pons, and optic chiasm appeared normal.

Microscopic Examination: Blocks of tissue from corresponding areas on each side of the formalin-fixed brain were taken for study both by frozen and paraffin sections. Hematoyxlin and eosin, eosin-

methylene blue, phosphotungstic acid hematoxylin, Nissl's stain, Pal-Weigert's, and Loyez and Perdrau's stains were made on the paraffin sections. Gold chloride sublimate, Penfield's combined method for oligodendroglia and microglia, Bielschowsky's, and Scharlach R. stains were made on the frozen sections.

Only in the abnormal portion of the right frontal and parietal lobes were changes of the leptomeninges found. The outstanding lesions were: (1) the presence of nests of astrocytes in the subarachnoid space connected with the brain through the defect in the pia mater; (2) the marginal gliosis; and (3) the lack of, or a partial myelinization of the nerve fibers in the white matter with a resulting extensive gliosis. No abnormal changes of the blood vessels were noted other than that they were increased in number in the white matter and scarce in the cortical gray matter. The neuronal elements in every part of the brain appeared unchanged.

The nests of astrocytes made up what in the gross appeared as the translucent gray-white localized thickenings of the leptomeninges. In many sections a connection between the cortex and the islands of tissue in the subarachnoid space was seen (Fig. 6). This connection appeared as a narrow break in the pia mater. There was no streaming of astrocytes into this subarachnoid tissue as is seen in a small hernia or an adhesion formation following injury to the leptomeninges. Again, there was no increased vascularity or growth of blood vessels toward or from the meninges such as is seen in a reaction to injury. There were many protoplasmic, lesser numbers of fibrous astrocytes, few neuroglia fibrils and nests of what appeared to be astroblasts. The latter had, in places, definite relations to blood vessels. In some areas they were closely approximated to the outer wall (Figs. 4 and 5). In other areas they occurred in long clumps apparently unconnected with blood vessels. There was very little connective tissue reaction in the leptomeninges, and this was always about the islands of astrocytes (Fig. 6). The significance of the astrocytes in this position is not known. They are localized in the only gross abnormal part of the brain where there are small defects of the pia mater. Nerve cells, nerve fibers and microglia or oligodendroglia were not demonstrated in these areas in the leptomeninges.

The marginal gliosis was present only in the abnormal areas. It corresponded with the thin white cortical zone seen beneath the

leptomeninges in the gross. This gliosis was not seen at the site of the defect in the pia mater where the cortex and the subarachnoid nests are connected.

In the white matter of the involved areas, the usual closely packed nerve fibers were partially replaced by a dense gliosis, unmyelinated fibers and occasional small areas of hemosiderin deposits. There was no subependymal reaction.

The neuronal elements were normally arranged, and no abnormalities of these cells were observed.

Many unsuccessful attempts to stain microglia and oligodendroglia in the abnormal areas were made but they were not demonstrated. They were successfully stained in other parts of the right hemisphere and left hemisphere. The significance of the lack of demonstration of these elements cannot be discussed as they may well be present and not brought out due to personal errors in technique.

In no section of the brain was there found an abnormality of the blood vessels, cell reaction about the blood vessels or in the cerebral tissue other than the gliosis mentioned above. No large or atypical ganglion cells were seen.

Comment

This case is an example of a cerebral tumor suspect in an infant with a history of convulsions and the development of an asymmetrically enlarged head. The ventriculograms were interpreted as showing a tumor compressing the posterior horn of the right lateral ventricle. The patient was then transferred to this clinic for the purpose of making an exploratory craniotomy. However, an intervening respiratory infection proved too much, and death occurred shortly after admission.

At necropsy and before the brain was sectioned, the lesion was taken to be a tumor of some unusual sort. The error was obvious after sectioning the brain.

The reason for the lack of development of the right frontoparietal area was not found. As far as could be determined there was no abnormality of the larger cerebral arteries. In the absence of any evident block in the ventricular system the asymmetrical hydrocephalus could not be explained. The aqueduct of Sylvius was patent, and the fourth ventricle was of normal size.

Without the complete autopsy a classification other than a localized abnormality of development will not be attempted. At this point attention may be drawn to the associated polydactylism. The defects of myelinization and the marginal gliosis only in the abnormal area are similar to the lesions seen in some forms of cerebral sclerosis. There are no subependymal areas of gliosis forming projections such as are so frequently described among the lesions found in cases of tuberous sclerosis.

In addition to the similarity of the lesion to a tumor the multiple, local areas of incomplete development of the leptomeninges and the presence of nests of astroblasts and astrocytes in the subarachnoid spaces of these areas are of considerable interest. These nests of cells of the astrocyte type are unusual findings. The cells are stained specifically by gold chloride sublimate and show up well with the phosphotungstic acid hematoxylin preparations. Glia fibers are present in small amounts, and only very few fibrous astrocytes are seen. Microglia and oligodendroglia could not be demonstrated in the nests, and no nerve cells or nerve fibers were seen. The lesion was not suggestive of any stage of an inflammatory process. The nests of cells resembled somewhat those found by Wolbach [1] as multiple, scattered nests of astrocytes in the subarachnoid spaces of spinal cord. Wolbach made multiple serial sections and proved that there was no connection between these nests and the spinal cord. The nests of cells described here differ from those he described in that they are connected with the cortex and that by specific stains astroblasts and astrocytes are demonstrated. The presence of astroblasts suggests the formation of the astrocytes in the leptomeninges of this region. However, were there no connections of these nests of astrocytes to the cortex the suggestion that the astrocytes present in the leptomeninges were formed from ectodermal elements derived from the neural crest might be offered, not only to interpret their presence but also to support the view of Harvey and Burr [2] as to the origin of the leptomeninges.

Conclusions

A localized abnormality of the right cerebral hemisphere was found after death in the brain of an infant suspected during life of having a brain tumor. The lesion, when disclosed, was thought to be an unusual tumor until the brain came to be sectioned.

Had this lesion been exposed at operation it might easily have been mistaken for an enucleable tumor, and had a fragment of one of the localized thickenings of the meninges been taken for immediate diagnosis the tissue might well enough have been reported as a glioma.

REFERENCES

1. Wolbach, S. B. Congenital rhabdomyoma of the heart. Report of a case associated with multiple nests of neuroglia tissue in the meninges of the spinal cord. *J. Med. Res.*, 1907, xvi, 495.

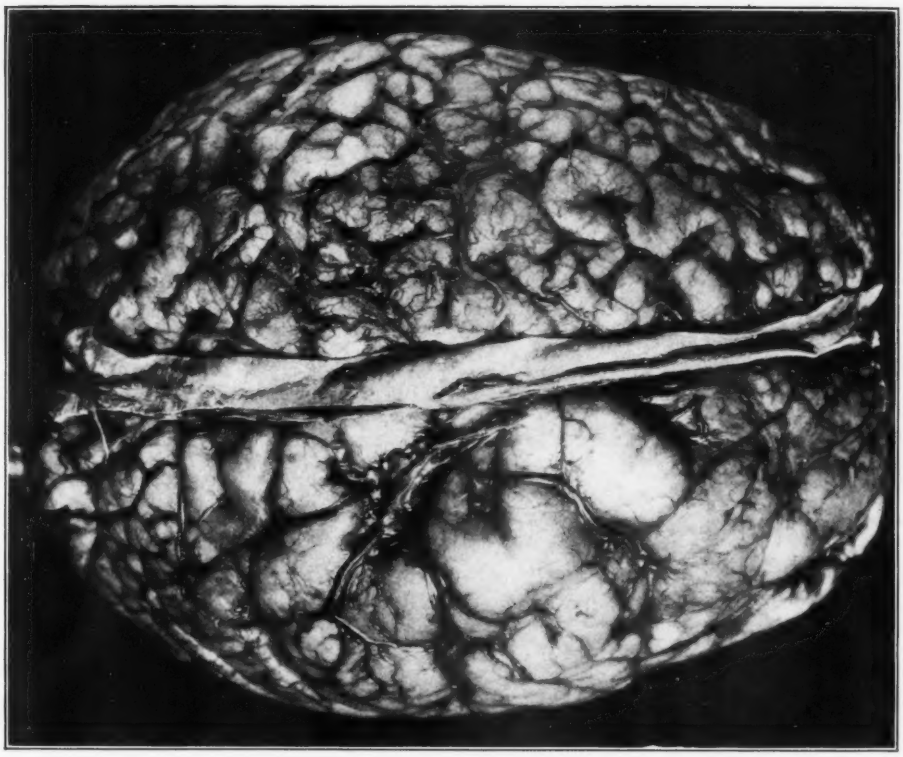
2. Harvey, S. C., and Burr, H. S. The development of the meninges. *Arch. Neurol. & Psychiat.*, 1926, xv, 545.

DESCRIPTION OF PLATES

PLATE 93

FIG. 1. A view of the superior surface to show the abnormality of the right frontoparietal lobes. The macrogyri and large dilated blood vessels at the posterior portion of the abnormality are well brought out.

FIG. 2. A portion of the leptomeninges has been removed from the frontal poles to show better the contrast between the normal left side and the abnormal right side. Irregular areas of thickening of the leptomeninges are evident over the abnormal portion which appears to be enucleable.



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Buckley and Deery Abnormality of Cerebrum and Leptomeninges

Plate 94

Fig. 3. This section is taken from in front to show the dilated right ventricle with the dam-like structure within. The right hemispheres show well the abnormality of gyri and the gray-white marginal zone of gliosis. The apparent anomaly in the wall of the left ventricle was produced by postmortem inflation of the ventricles with air.

Fig. 4. A frozen section, stained with gold chloride sublimate, through a local thickening of the leptomeninges. The clusters of astrocytes stream along parallel with the cortex. There is no evidence of a pia mater. × 175.

3

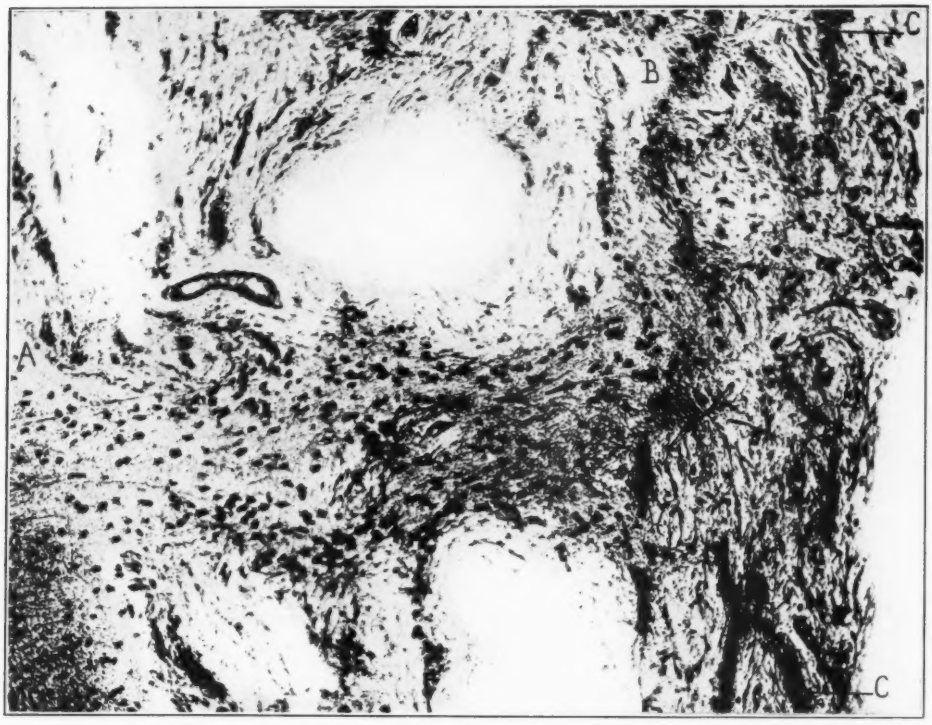
4

Buckley and Deery Abnormality of Cerebrum and Leptomeninges

PLATE 95

FIG. 5. Section through the thickened leptomeninges and cortex to show the connection between the two. (*a*) There are multiple nests of astrocytes (*b*) even near the under surface of the pia arachnoid. Many of the cells in these nests resemble astroblasts and are closely approximated to blood vessels (*c*). Gold chloride sublimate. × 175.

FIG. 6. Section through the leptomeninges and cortex stained by Perdrau's method to show the defects in the pia mater and the connections between the cortex and the glial tissue in the leptomeninges. The nuclei of the astrocytes are shown by this stain. There is no connective tissue shown in the areas of astrocytes in the leptomeninges. × 110.



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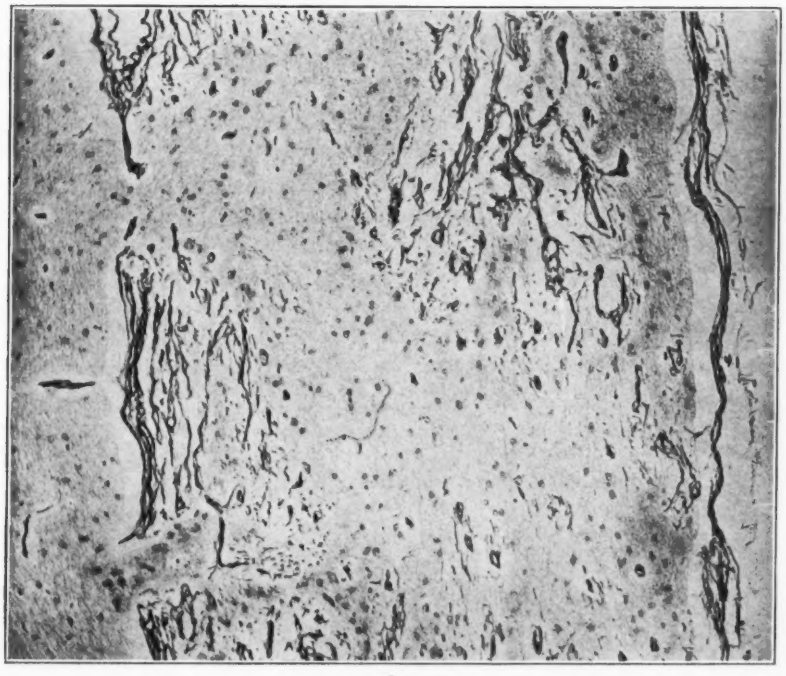

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Buckley and Deery Abnormality of Cerebrum and Leptomeninges

TISSUE CULTURE STUDIES OF THE GLIOBLASTOMA MULTIFORME *

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As a part of the examination of each intracranial tumor verified at operation in this clinic, tissue culture preparations are made by the hanging drop method [1] in order, if possible, to further our knowledge of the histiogenesis of the tumors.

Growths have been observed in the cultures of certain of the gliomas, acoustic nerve tumors, metastatic epithelial tumors, adenomas of the hypophysis and ependymal tumors. However, the best results and the most consistent in type of growth are obtained from the operative specimens of the glioblastoma multiforme,† a rapidly growing invasive glioma of the cerebral hemispheres. This tumor is the most common type among the 496 verified and classified gliomas studied in this clinic. Up to February 1, 1929, there were 160 verified examples of this type of glioma. From ten to fifteen of these tumors are available in this clinic for study each year.

The purpose of this report is to present the results of the cultures made from the tissue of tumors diagnosed as glioblastoma multiforme, ten of which have been studied by the tissue culture method.

Of these ten separate tumors, five showed excellent growth *in vitro*, but no comparable growths were obtained from the remaining five although the same technique was used. Although many difficulties are met with in tissue culture preparations, it did seem that the lack of growth could be explained in some other manner. When the clinical histories and operative findings of the ten cases were studied, they were found to be similar to those of previously verified cases of the same type of glioma. But when histological studies of the paraffin sections were made there was found a considerable variation in cell type and arrangement. The histological sections of the five cases showing excellent growth in culture showed quite similar

* Received for publication June 7, 1929.

† These tumors were formerly termed spongioblastoma multiforme, but in accordance with the suggestion of Bailey and Cushing [2] the designation glioblastoma is now employed as more properly descriptive.

cell types and architecture. They were invasive, rapidly growing, with many areas of necrosis. The majority of the cells were those with a single large nucleus, a pyriform or fusiform-shaped body and either one or two processes. They resembled spongioblasts. Large multinucleated cells were frequently seen, but astrocytes were less common. Mitotic figures were abundant. The diagnosis accepted in each case was a glioma of the glioblastoma multiforme type. The histology of the remaining five tumors from which poor or no growths were obtained, varied. They were interpreted as rapidly growing gliomas with cells differentiating into astrocytes; probably of the glioblastoma multiforme type. That certain of these diagnoses were later changed is shown by the fact that one year postoperative the necropsy specimen of one tumor was considered as a typical astroblastoma. The predominating cell in these sections was of the protoplasmic astrocyte type with occasional giant cells and pyriform or fusiform cells with one or several processes.

Before considering the characteristics of the successful cultures the following history is presented. It will serve as an example of the known life history of any of the tumors that grew in culture as well as an illustration of the methods of examination of brain tumor tissue removed at operation.

Surg. No. 29667. Philip T., aged 42 years, dated the onset of his present illness to July 16, 1927, when a sudden twitching of his facial muscles occurred. One month later a left brachial monoplegia and signs of increased intracranial pressure developed. Two months from the onset of his illness he was admitted to this clinic, and a diagnosis of a rapidly growing glioma of the right parietal region was made — "most probably a glioblastoma multiforme." At operation, what was regarded by Dr. Harvey Cushing as grossly a typical spongioblastoma multiforme was partially removed by suction. During the operation a part of the tissue was sent to the laboratory for purposes of supravital preparations and tissue culture. A study of the immediate supravital preparations, made by Dr. L. S. Kubie, supported the gross diagnosis. The tissue was very cellular with the majority of the cells being interpreted as spongioblasts. The multinucleated cells and fibrils were less plentiful. Growths in many separate preparations of the same tissue studied by the supravital technique were observed over a period of ten days, subcultures being made. Morphologically, the growing cells resembled those seen in the supravita[1] smears.

Within a week paraffin sections stained by the eosin-methylene blue and Mallory's phosphotungstic acid methods were available. The structure of the tumor was that of a rapidly growing glioma with many areas of necrosis and a varied type of cells with many mitoses, typical of the glioblastoma multiforme.

From any of the above studies a poor prognosis was offered. Extensive X-ray therapy was given. However, a recurrence of his symptoms was reported; and

within fourteen months from the onset of his symptoms death occurred at his home. A necropsy was not performed.

The tissue culture preparations from each of the five separate tumors diagnosed spongioblastoma multiforme were made within thirty minutes of the time of removal at the operation. The best preserved and most cellular appearing tissue was used. Observations of the cultures were made in a warm box adjacent to the incubator. Attempts at photomicrography of the living cells were in general unsuccessful and resulted in the loss of some preparations. So the preparations were fixed at intervals in 5 per cent formaldehyde, formalin ammonium bromide, and Zenker's solution. Photomicrographs were then made of the fixed, unstained and stained preparations. The fixed sections were stained with methylene blue, hematoxylin and eosin, gold chloride sublimate, and silver carbonate.

Remarkable similarities in the arrangement and type of the cells and the methods of cell division were seen in each of the growing preparations. These features will be taken up separately.

The pattern of growth is shown well in Figs. 1 and 2, where the suggestion of a rapidly growing, invasive type of tumor is evident. Such a cell growth has not been seen in the other cerebral tumor tissue cultures. Although in many preparations, individual cells were seen jutting out from the periphery of the explant within several hours of culturing, the growth in each culture was usually apparent from twelve to eighteen hours after planting. It is possible that some of these cells were mechanically displaced in culturing and then extended out. In addition to these definite cell bodies (Figs. 1, 2, 4, 6) extending out from the periphery, there were frequently seen small, rounded, finely granular anuclear bodies, varying in size, surrounded by a shining membrane, the whole being attached to the edge of the explant or to the end of a process coming out from the explant (Figs. 2, 3, 7). They were also found attached to cells and their processes out in the field of growing cells. Their significance is not known. They were not seen in the cellular portions of the paraffin sections of the same tumors. Actual cell formation from these bodies was not seen and they did not appear to be dying or dead cells.

Soon extension of the cells into the media was seen. The method of outgrowth, the connection of the cells and the type of cells were similar in the different cultures (Figs. 2, 3, 4). In some fields (Figs. 1, 4, 6) cells with fusiform bodies, a single nucleus and two long, taper-

ing processes predominated. The nuclei of these cells varied in position. At the edge of the explant where the cell body was pushing into the media and there was no process, the nucleus was seen at the advancing, blunted pole of the cell body. Among the growing cells the nuclei of the same cells were situated in the center or at one pole. The granules closely packed the cell bodies and extended out into the processes for varying lengths. Often there were local swellings in the processes caused by the accumulation of granules.

Connected by long, thin or thick processes with the above described cells there were many irregular, gigantic, multinucleated cells (Figs. 2, 4, 5). As many as seven nuclei have been counted in these large cell bodies. The method of formation of these large cells was not determined. A fusion of small cells to form a giant cell was not seen.

Occasionally cells different from the above grew out from the explant. These cells are the only type resembling astrocytes with the specific stains seen in any of the tissue cultures (Fig. 4). They have irregular bodies and a large, oval, vesicular, eccentrically placed nucleus. A nucleolus was not observed in these cells. They are made star-shaped by from 4 to 7 processes which extend out in any direction to be attached to a process of another cell of any of the above types. Fibrillae could not be demonstrated in their cell bodies or processes.

These various cell types are seen in the cultures fixed at from 24 to 144 hours after culture. In the few successful subcultures the pattern of growth and cell types resembled, in the living and fixed preparations, the original cultures. Just how long cultures of these tumors could be kept alive by this technique is not known. In the series the longest preparation was kept ten days. When it had extended to the vaseline ring on the cover slip it was fixed and stained.

Cell division by mitoses was commonly seen in the cultures and growths. Regular mitotic figures in the fusiform type of cells with a single nucleus were observed at the edge of the explant and out among the growing cells. In Fig. 3 the end result of mitosis is seen as the two separate cell bodies are dividing. Irregular mitoses were seen in the star-shaped and multinucleated cells (Figs. 4, 5). Wherever mitotic figures were present the cell processes were much thicker than in the apparently non-dividing cells. No other form of cell division was seen.

A fairly close resemblance in cell types was noted in comparing the living and fixed stained tissue cultures with the supravital preparations and the stained paraffin sections. The various cultured cells, as illustrated, were well impregnated by the gold chloride sublimate and the silver carbonate stains. The cells were less intensely stained by methylene blue and hematoxylin and eosin. Attempts at other and more complicated stains were unsuccessful.

From these observations, the most common type of cell growing in the cultures of the tumors diagnosed as glioblastoma multiforme is that with a fusiform or pyriform cell body, an oval nucleus, an occasional nucleolus, with a process extending out from one or both ends of the cell body. The granular cytoplasm of the cell body is continuous with the processes. These cells resemble very closely unipolar and bipolar cells, forming the most common cell in a successful culture of a medulloblastoma in which the cells appear to be differentiating into spongioblasts. The less commonly found cells were the multinucleated ones and those resembling astrocytes. The mitoses were of regular formation in the small and common type of cells.

Summary

Tissue culture preparations of ten intracranial gliomas removed at operation were made. The clinical histories and the gross structures of these tumors as seen at operation resembled previously verified examples of glioblastoma multiforme.

The cultures of five of these tumors showed excellent and uniformly similar growths. The cultures of the other five tumors presented none or very little growth.

The histological structure seen in the paraffin sections of the tumor tissue from which a growth was obtained was interpreted as similar to that of the glioblastoma multiforme. A different structure in the other five tumors made the diagnosis difficult. They were interpreted as a rapidly growing glioma with cells differentiating into astrocytes.

There was noted a striking similarity in the resemblance of the growing cells to those seen in fresh smears and stained paraffin sections of the same tumor tissue.

REFERENCES

1. Lewis, W. H., and Lewis, M. R. The behavior of cells in tissue cultures. General Cytology, Section vii, 385–391. University of Chicago Press, 1924.
2. Bailey, P., and Cushing, Harvey. Classification of the Tumors of the Glioma Group. J. B. Lippincott Co., Philadelphia, 1926, 95.

DESCRIPTION OF PLATES

PLATE 96

FIG. 1. S 29667. Cell growth of a culture of glioblastoma multiforme fixed at ninety-six hours, the cells extending out to the edge of cover slip. Gold chloride sublimate. × 80.

FIG. 2. S 29667. Edge of explant of culture of glioblastoma multiforme at end of forty-eight hours, demonstrating variety of cells. (*a*) Multinucleated cell, (*b*) fusiform cell with mitosis and thickened process, (*c*) cell "buds." Unfixed, unstained. × 300.

1

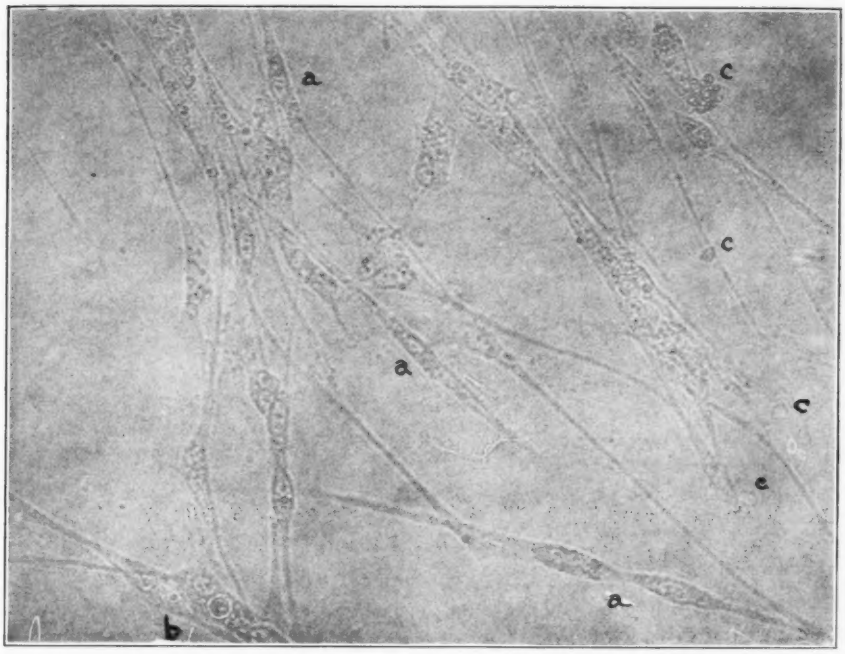
2

Buckley Glioblastoma Multiforme

PLATE 97

FIG. 3. S 29667. Outer field of growing cells of culture of glioblastoma multiforme showing at (*a*) apparent division at end of mitosis of spindle-shaped cell, at (*b*) multinucleated cell and cell "buds" at (*c*). Unfixed, unstained. × 300.

FIG. 4. S 30556. Edge of explant of culture of glioblastoma multiforme showing multinucleated cells, mitotic figures, cell buds and cells resembling astrocytes. Gold chloride sublimate. × 225.



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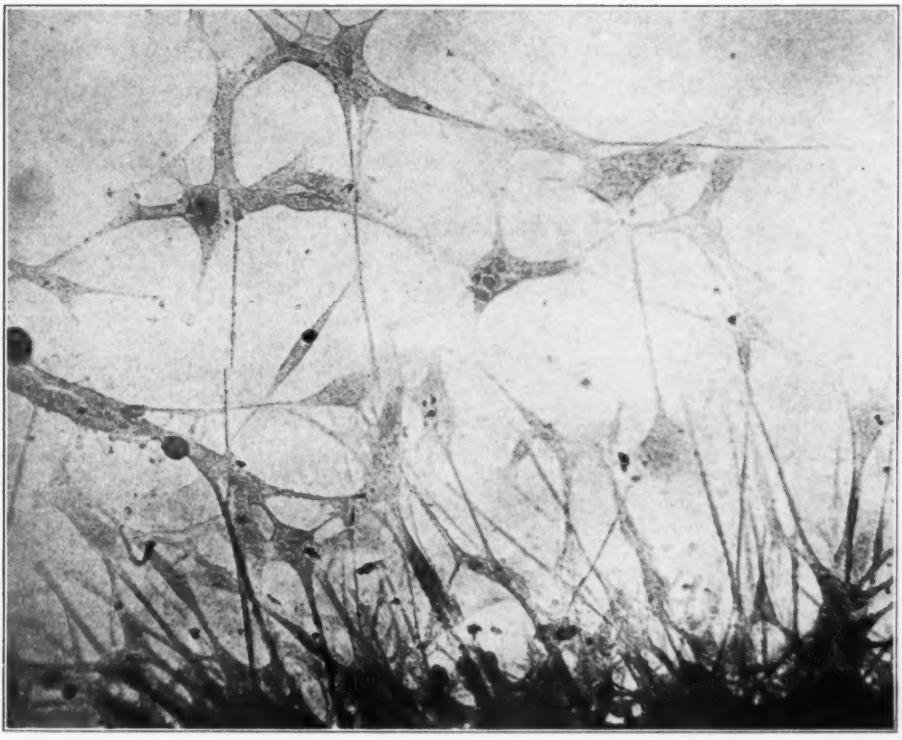

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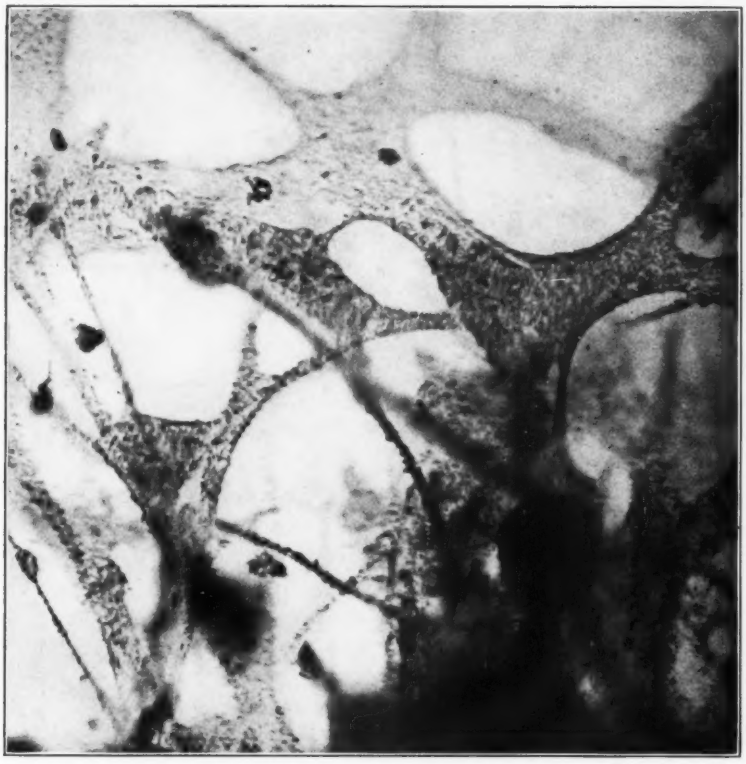
Plate 98

Fig. 5. S 30556. Mitotic figures in large cells of a culture of glioblastoma multiforme. Gold chloride sublimate. × 850.

Fig. 6. S 30556. Cell of spongioblast type at edge of explant of culture of glioblastoma multiforme. Gold chloride sublimate. × 850.

Fig. 7. S 29667. Large cell bud and irregular mitotic figures in lower cell of a culture of glioblastoma multiforme. Gold chloride sublimate. × 850.



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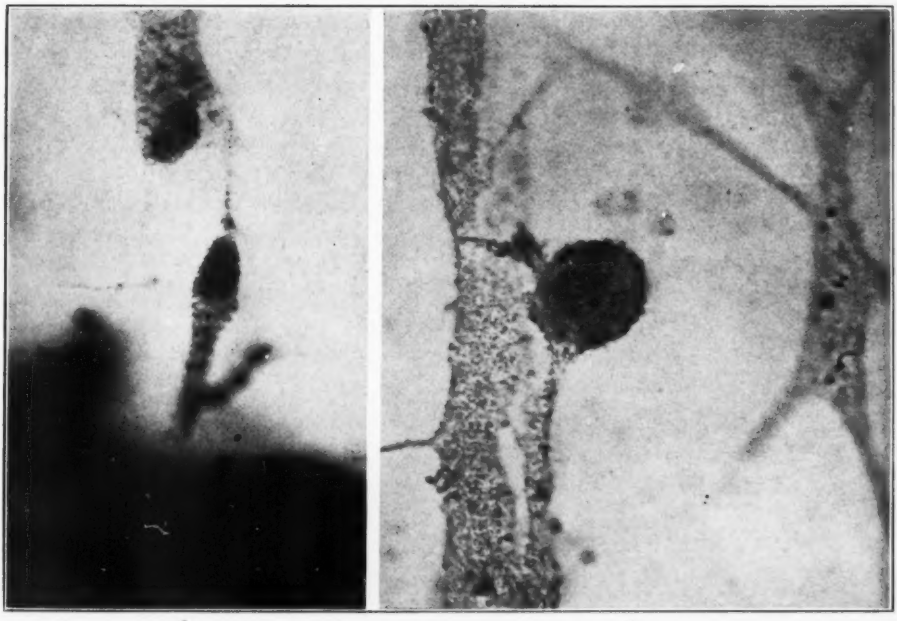

6 7

Buckley Glioblastoma Multiforme

THE PRODUCTION OF ALLERGIC INFLAMMATION IN THE KIDNEYS *

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INTRODUCTION

Since Arthus [1] first described the phenomenon in 1903, much work has been done upon the local reaction which occurs in the subcutaneous tissues after repeated injections of a foreign protein. Little notice, however, has been given to the occurrence of a similar phenomenon in internal organs. Friedberger [2] produced a cellular pneumonia in sensitized guinea pigs by spraying horse serum into the trachea. Ishioka [3] has reported and confirmed these experiments. Schlecht and Schwenker,[4] after careful histological study of the lungs in this condition, described the proliferation of the cells lining the alveolar walls, and the exudation of eosinophilic leucocytes into the alveoli with the formation of fibrin which gives rise to a type of bronchopneumonia. Opie [5] observed a localized consolidation surrounding a central focus of necrosis with leucocytic infiltration and edema after injecting 0.2 cc. of horse serum through the thoracic wall into the lung of a sensitized rabbit. Shapiro and Ivy [6] produced ulcers of the stomach by injecting foreign protein into the gastric mucosa of sensitized rabbits and dogs. Long and Seyfarth [7] found only a moderate and transient interstitial infiltration of wandering cells after the introduction of egg albumin and gliadin into the testicle of guinea pigs sensitized to these proteins. The mild mononuclear infiltration which resulted did not definitely exceed the similar response to intratesticular injection of these same proteins into normal guinea pigs. As far as we know there has been no work done on anaphylactic inflammation in the kidneys of sensitized animals. Long and Finner [8] attribute a coagulation necrosis of the glomeruli and tubules found in one kidney of a tuberculous pig four days after perfusion with tuberculin protein to anaphylactic inflammation. Longcope [9] and Boughton [10] have reported an experimental nephritis in sensitized animals by repeated proteid intoxication. This, however, was produced by anaphylactic shock and not as a local reaction of the renal tissue.

* Received for publication May 20, 1929.

Arthus [1] injected rabbits subcutaneously with a foreign protein, every six days and found that local toxicity of the protein steadily increased. The material of the first three injections was absorbed within a few hours; the fourth produced a soft infiltration of the skin which remained two to three days; the fifth a hard infiltration which remained five or six days; the sixth an extensive damage to the subcutaneous tissue which was transformed into a compact, white aseptic mass which remained unchanged for weeks; the seventh a superficial necrosis which terminated in a cutaneous ulcer that healed only after several weeks. The onset of this phenomenon is not dependent upon the number of injections entirely, as there are individual variations and a greater number of injections may be necessary. The reaction is not the result of the amount of protein injected, as only 0.5 cc. of the solution gave a strong reaction, but only in specifically sensitized animals. The preliminary sensitization could be made by any parenteral route. The severity of the local reaction differs with the location of the tissue injected. The reaction occurs most markedly in the subcutaneous tissues of the chest and abdominal wall, while only an edema occurs on the ear.

Arthus and Breton [11] studied this subcutaneous reaction microscopically: Twenty-four hours after injection of the homologous protein into a sensitized rabbit there was edema with many leucocytes, and the connective tissue fibers were doubled in volume. After forty-eight hours this condition was more extensive with hemorrhage in the deeper layers of the skin down to the muscle; this remained stationary for about two days, then the skin sloughed off and there resulted an ulcer, the floor of which was covered with a soft, cheesy, sterile mass composed of disintegrated leucocytes. Gerlach [12] described an earlier stage: Within the first hour there develops a rapidly spreading edema; after an hour there is a marked swelling of the connective tissue fibers with compression of the capillaries causing an anemic necrosis in the central portion; surrounding this area there is edema with precipitation of fibrin, hyperemia, and later stasis in the vessels which became plugged with white blood corpuscles; there is also a massive emigration of leucocytes. Anaphylactic inflammation is characterized by its rapid course and the intensity of the reaction. Rössle [13] speaks of this augmented reaction in the tissues as "the highest inflammation-capacity of the organism."

Opie [14] thinks the above necrosis and inflammation due to the oc-

clusion of the capillaries and small vessels by hyaline thrombi, formed by the reaction of the antigen with its antibody, the leucocyte infiltration being caused by the positive chemotaxis of the precipitate formed. Metalnikow [15] showed that in sensitized animals the antigen possessed positive chemotactic activity. Coca [16] has suggested that the necrosis might be caused by muscular contraction of the arteries. Klinge [17] demonstrated that it was not due to a sensitized nervous system.

Rössle,[18] by using an antigen that could be recognized in microscopic sections, *viz.*, chicken blood, found that it was absorbed much more slowly and with greater difficulty in sensitized than in normal rabbits. The more frequent the injections, the more difficult the resorption; the injected cells at the site of the injection disintegrated locally. In normal animals ordinary leucocytes predominated in the resorptive inflammation, while in anaphylactic inflammation the eosinophiles early predominated.

Auer [19] found that it was not necessary to inject the specific protein locally to produce inflammation. Xylol applied to the ears of a sensitized rabbit which was reinjected intraperitoneally with the antigen, produced a local gangrene by concentrating the antigen in the ears. "A subminimal concentration of antigen for non-inflamed sensitized cells may thus become effective when inflamed sensitized cells are concerned." He suggested that this may theoretically occur in any tissue of a sensitized animal which can show an anaphylactic reaction and that these conditions might explain a number of functional abnormalities in the human subject. Tannenberg,[20] however, using Auer's method, obtained a marked diminution of the inflammatory changes after applying cantharadin to the backs of sensitized rabbits.

Nicolle [21] demonstrated in 1907 that this local inflammation was of an anaphylactic nature in that it occurred in animals that were passively sensitized. It is, therefore, dependent upon specific substances transmissible in the serum.

Opie [5,22] has done extensive work on anaphylactic inflammation in the light of modern conceptions of anaphylaxis. He contributed the following: (1) the reaction depends on the simultaneous presence of antigen and antibody in the tissues; (2) the severity of the reaction varies with, but does not exactly parallel, the precipitin titer of the serum; (3) the reaction varies in different species; (4) the local reac-

tion protects the animal from a general reaction by limiting the antigen to the site of injection; (5) a total desensitization of the tissues and disappearance of the precipitin follows the response to a pure antigen such as crystalline egg albumin, but not to a complex one as egg-white or horse serum.

Grineff [23] described a therapy for anaphylactic inflammation. He found that intravenous injections of small quantities of a foreign protein prevented the subcutaneous reaction to the local injection on the following day.

Wells [24] and Opie [14] have suggested, as has Auer, [19] that anaphylactic inflammation might be produced in other tissues than the skin under similar conditions.

Method

The proteins used in these experiments were commercial horse serum (Parke, Davis and Co.) and a 2 per cent solution of crystalline egg albumin (Merck) made fresh each time in Ringer's solution. Rabbits were used for all the experiments and were sensitized by the original method of Arthus. The subcutaneous injections of 1 cc. of horse serum or 2 cc. of egg albumin were made into the abdominal wall. The injections were every five or six days, alternating the side each time. They were continued until extensive necrosis of the skin was obtained which assumed a purplish black color. The next injection, five days later, was made directly into the left kidney. To do this an assistant holds the rabbit in a flexed position; the kidney is grasped through the abdominal wall and is brought up and held firmly immediately under the skin. With a small hypodermic needle and under aseptic conditions, 0.5 cc. of the protein is injected directly into the kidney. The feel of the needle as it passes through the renal capsule indicates that the injection is being made into the substance of the kidney. The kidneys were injected from one to eight times at five or six day intervals. The rabbits receiving from one to four injections in the kidney were killed twenty-four and forty-eight hours after the last injection. Four groups of rabbits received five six, seven, and eight injections respectively, in the kidney, and animals from each group were killed at intervals of forty-eight and seventy-two hours, one, two, and three weeks and one month. The right kidney of each rabbit was used as a control, except in eight cases. In these rabbits the left kidney was injected three times and at

the same time as the last injection, the right kidney was injected and the animals killed in twenty-four and forty-eight hours. At autopsy, samples of urine were obtained from the bladder with which albumin tests and microscopic examinations were made.

At the time of the first injection in the kidney, blood was taken from the ear for precipitin tests. The serum was run to progressive dilutions of antigen by the ring method, the tubes standing in a water bath at 37° C for one-half hour.

Control rabbits received one to eight intrarenal injections and were killed twenty-four and forty-eight hours after the last injection in the left kidney. Seven were injected with 0.5 cc. of a sterile 2 per cent solution of Silver Label gelatin in Ringer's solution. Eight were injected with egg albumin without being previously sensitized. One was injected with India ink in both kidneys and killed after the right had been injected thirty minutes and the left one hour.

Results

The average number of injections of horse serum required to produce necrosis of the skin was seven; of egg albumin, six. Eight rabbits having from thirteen to twenty-one subcutaneous injections showed a very slight reaction. This confirms Arthus' work that all animals do not react to the same degree. Four rabbits that had been sensitized to horse serum one year before showed a marked skin necrosis after the second injection. The horse serum and the egg albumin produced the same type of reactions in the kidneys.

The results of the precipitin titrations agreed with those of Opie's mentioned above, namely, that the severity of the reaction varies with, but does not exactly parallel, the precipitin titer of the serum.

Of fifty-four sensitized rabbits, thirty-two were killed within seventy-two hours after the last injection in the left kidney, and twenty-two after seventy-two hours. Twenty of the thirty-two rabbits killed within seventy-two hours showed hemorrhage associated with necrosis at the site of injection in the left kidney, seven in the right kidney. One killed in four days showed a small hemorrhage. The fact that the red cells were all well preserved was evidence that the hemorrhage was due to the last injection. Six not included in the twenty showed hemorrhage beneath the renal capsule only. One of the right kidneys injected had a hemorrhage beneath the capsule only. Six

showed no evidence microscopically of having been injected in the kidney.

TABLE I

Hemorrhage and Necrosis in Sensitized Rabbits

Number of rabbits	Left kidney		Right kidney
	Autopsy within 72 hrs.	Autopsy after 72 hrs.	Autopsy within 48 hrs.
Sensitized	32	22	8
Showing hemorrhage and necrosis	20	1	7
In pyramid and medulla	14	1	2
In cortex	6	0	5
With hemorrhage underneath capsule	6	0	1
Not showing hemorrhage	6	21	0
With recent scar tissue	8	22	0

No recent hemorrhage was observed in the rabbits killed one or more weeks after the last injection. The injected kidneys of all of these had some recent scar tissue, or hemosiderin was present in necrotic areas undergoing organization. Eight that were killed within seventy-two hours and showed recent scar tissue had had at least three injections in the kidney, the scar tissue resulting from an earlier injection.

When the hemorrhage occurred in the medulla or pyramid, the amount of necrosis exceeded that of hemorrhage. The areas of hemorrhage and necrosis in this part of the kidney had a very definite outline. That the necrosis was due chiefly to the reaction of the sensitized tissues to the antigen is shown by the absence of necrosis around the hemorrhages in the control rabbits. The definite outline of the necrosis may have been related to the less highly specialized tissue of the medulla and pyramid; or possibly, to peculiarities of the histologic architecture of this portion of the kidney.

The hemorrhage and necrosis was greatest when the protein was injected into the cortex and the animal killed in twenty-four hours. This necrosis did not surround the hemorrhage evenly but spread out irregularly from all sides, but most markedly toward the capsule. That the material injected did not diffuse out in the surrounding tissue to any great extent was demonstrated in the kidneys injected with India ink. The kidneys with areas of necrosis had had from one

to three injections, except one kidney which had received seven injections. Those that showed a large amount of necrosis without hemorrhage in the cortex had had five injections, the necrosis being the result of a previous injection, as there was a definite increase in the connective tissue.

In the necrotic areas of the cortex, the epithelium of all types of tubules was swollen and showed necrosis. Few of the glomeruli were necrotic, most of those in and adjacent to the area of necrosis were distended with blood. The connective tissue was affected least, sometimes only showing edema in an area of necrosis, and soon started to proliferate. There was a variable infiltration of leucocytes in and around the area of necrosis; only a few were eosinophiles. Hyaline and blood casts were found in most of the collecting tubules near this area. In all the kidneys except two, the necrosis was of the coagulation type. In these two there was liquefaction necrosis due to an extensive infiltration of polymorphonuclear leucocytes.

The following protocols illustrate the results:

RABBIT NO. 2

Feb. 1 to Apr. 19: Received twenty-one subcutaneous injections of horse serum in the abdominal wall, had a two plus skin reaction,* and a precipitin titer of 40,000.

Apr. 23: Injected 0.5 cc. of horse serum into the left kidney.

Apr. 27: Injected 0.5 cc. of horse serum into the left kidney.

Apr. 28: Autopsy.

Urine: None obtained.

Description of Left Kidney; Gross: Two needle punctures were found, one containing a blood clot which on the cut surface extended into the substance of the kidney for about 7 mm.

Microscopic: A large hemorrhage extends from the cortex to the pyramid. In an area at one side of the hemorrhage extending from the medulla up to the cortex, there is marked necrosis of the tubules and a slight edema. A few of the glomeruli nearest the hemorrhage are necrotic, and a few are distended with blood. There is only a slight infiltration of leucocytes in the necrotic area, but a larger number surround the hemorrhage. There are a great many hyaline casts in the collecting tubules in and near the area of necrosis.

* One plus represents a slight edema for twenty-four hours; two plus, greater edema lasting for five days, then disappearing; three plus, hard fibrotic induration lasting a much longer time; four plus, necrosis with ulceration.

Description of Right Kidney: Grossly and microscopically normal.

RABBIT NO. 101

Dec. 17 to Jan. 13: Received five subcutaneous injections of egg albumin in the abdominal wall, had a four plus skin reaction, and a precipitin titer of 5000.

Jan. 17: Injected 0.5 cc. of egg albumin into the left kidney.

Jan. 21: Injected 0.5 cc. of egg albumin into the left kidney.

Jan. 25: Injected 0.5 cc. of egg albumin into the left kidney and 0.5 cc. into the right kidney.

Jan. 26: Autopsy.

Urine: albumin, positive; microscopic, few hyaline and many granular casts, few white blood cells.

Description of Left Kidney; Gross: The upper pole had a small hemorrhage underneath the capsule and a pale area on the surface of the kidney at the site of an injection. The lower pole was larger than normal and hard. A large pale area covered the central and lower parts of the kidney. The cut surface appeared normal.

Microscopic: There is a slight hemorrhage along the needle puncture which terminates in a larger one in the medulla and pyramid. In the lower portion of the cortex at the beginning of the large hemorrhage there is necrosis of the tubules and a marked infiltration of leucocytes. There is also a marked infiltration of leucocytes at the lower edge of the hemorrhage. An occasional hyaline and blood cast was seen in the collecting tubules near the necrosis and many below the hemorrhage.

Description of Right Kidney; Gross: A small white area was found on the surface at the site of the injection; the whole kidney was paler than normal. The cut surface looked normal.

Microscopic: Above and to one side of a small hemorrhage in the lower part of the cortex, the tubules are necrotic and all the glomeruli in this area are affected. There is a slight edema and leucocytic infiltration above the hemorrhage with a greater infiltration of leucocytes in the more marked area of necrosis near the surface of the kidney.

RABBIT NO. 106

Dec. 17 to Jan. 13: Received five subcutaneous injections of albumin in the abdominal wall, had a four plus skin reaction, and a precipitin titer of 5,000.

Jan. 17: Injected 0.5 cc. of egg albumin into the left kidney.

Jan. 21: Injected 0.5 cc. of egg albumin into the left kidney.

Jan. 25: Injected 0.5 cc. of egg albumin into the left kidney, and 0.5 cc. into the right kidney.

Jan. 26: Autopsy.

Urine: albumin, strong positive; microscopic, few granular casts and many white blood cells.

Description of Left Kidney; Gross: Three needle punctures could be seen; the capsule was adherent over each one. The lower pole was hard, but not as hard as in Rabbit No. 101. The cut surface showed a hemorrhage along one needle puncture.

Microscopic: There is a slight hemorrhage near the surface of the kidney with necrosis of all the tubules surrounding this area and a moderate infiltration of leucocytes and fluid between them. The glomeruli around this area are distended with blood, two have been replaced by a mass of leucocytes.

Description of Right Kidney; Gross: The cut surface showed a hemorrhage in the pyramid. Otherwise the kidney was normal in appearance.

Microscopic: A long narrow hemorrhage (along the needle puncture) appears in the medulla. There is a very slight necrosis of the tubules surrounding the hemorrhage with a moderate infiltration of leucocytes.

Discussion

The inflammatory reaction in the sensitized animals was marked, whereas in the control rabbits there was only hemorrhage due to mechanical injury. The anaphylactic inflammation produced in the kidneys of these animals differed from other forms of inflammation. (1) In the rate of progress. In twenty-four hours a far advanced acute inflammatory reaction was present. Examinations at earlier stages have not been made. (2) In the relatively slight edema in the inflammatory areas in these kidneys as compared with similar areas in the subcutaneous tissues. This was probably due to the small amount of connective tissue in the kidney and to the effect of the dense capsule upon the increase of intrarenal pressure by relatively small exudates. The exudate appeared to consist chiefly of leucocytes with relatively little fluid. (3) In the extent of the necrosis which is primarily of the coagulation type. Liquefaction occurred, as in other forms of inflammation, only after infiltration of the area with polymorphonuclear leucocytes.

The cause of the necrosis is not entirely clear. Three possibilities suggest themselves. (1) Infarction due to thrombosis. Although thrombi were occasionally found, they did not appear to be numerous enough to account for the necrosis. (2) Gerlach believed that in areas of anaphylactic inflammation of the skin edematous swelling

of the connective tissue reached such a degree that compression of the capillaries and venules caused a prolonged ischemia followed by necrosis. The edema in the kidneys from these experiments does not appear to be sufficient to account for the necrosis. (3) The probable cause of the necrosis was the direct toxic effect of the antigen on the sensitized tissues. This is suggested by the fact that the more highly specialized and differentiated tubular epithelium of the cortex appeared to be more susceptible to injury than the less highly specialized epithelium of the collecting tubules of the pyramids. Opie [25] has shown that in sensitized animals the antigen remained localized in the tissues instead of being quickly reabsorbed as in non-sensitized animals. The antigen remaining concentrated at the site of the injection caused the necrosis and inflammation to be localized and more intense.

Conclusions

1. Hemorrhage was found in all the injected kidneys when the animal was killed within seventy-two hours.

2. Necrosis was observed in kidneys of sensitized rabbits only.

3. Anaphylactic inflammation differs from other inflammation in the intensity and rapidity with which it occurs.

4. This anaphylactic inflammation was caused by the direct toxic effect of the antigen on the sensitized tissues.

REFERENCES

1. Arthus, M. Injections répétées de sérum de cheval chez le lapin. *Compt. rend. Soc. de biol.*, 1903, lv, 817.

2. Friedberger, E. Die Anaphylaxie mit besonderer Berücksichtigung ihrer Bedeutung für Infektion und Immunität. *Deutsche med. Wchnschr.*, 1911, xxxvii, 481.

3. Ishioka, S. Zur Histologie der anaphylaktischen Pneumonie. *Deutsches Arch. f. klin. Med.*, 1912, cvii, 500.

4. Schlecht, H., and Schwenker, G. Ueber die Beziehungen der Eosinophilie zur Anaphylaxie. *Deutsches Arch. f. klin. Med.*, 1912, cviii, 405.

5. Opie, E. L. Inflammatory reaction of the immune animal to antigen (Arthus phenomenon) and its relation to antibodies. *J. Immunol.*, 1924, ix, 231.

6. Shapiro, P. F., and Ivy, A. C. Experimental production of gastric ulcer by local anaphylaxis. *Arch. Int. Med.*, 1926, xxxviii, 237.

7. Long, E. R., and Seyfarth MacH. The testicle as an indicator of allergy in the hypersensitiveness of infection and anaphylaxis. *Am. Rev. Tuberc.*, 1924, ix, 254.

8. Long, E. R., and Finner, L. L. Experimental glomerulonephritis produced by intrarenal tuberculin reactions. *Am. J. Path.*, 1928, iv, 571.

9. Longcope, W. T. The production of experimental nephritis by repeated proteid intoxication. *J. Exper. Med.*, 1913, xviii, 678.

10. Boughton, T. H. Kidney lesions in chronic anaphylaxis. *J. Immunol.*, 1916, i, 105.

11. Arthus, M., and Breton, M. Lésions cutanées produites par les injections de sérum de cheval chez le lapin anaphylactisé par et pour ce sérum. *Compt. rend. Soc. de biol.*, 1903, lv, 1478.

12. Gerlach, W. Studien über hyperergische Entzündungen. *Virchows Arch. f. path. Anat.*, 1923, ccxlvii, 294. Über Beziehungen der Entzündung zum anaphylaktischen Zustand. *Verhandl. d. deutsch. path. Gesellsch.*, 1923, xix, 126.

13. Rössle, R. Referat über Entzündung. *Verhandl. d. deutsch. path. Gesellsch.*, 1923, xix, 18.

14. Opie, E. L. Pathogenesis of the specific inflammatory reaction of immunized animals (Arthus phenomenon); the relation of local "sensitization" to immunity. *J. Immunol.*, 1924, ix, 259.

15. Metalnikow, S. Anaphylaxie et chimiotaxie. *Comt. rend. Soc. de biol.*, 1921, lxxxiv, 932.

16. Coca, A. F. The mechanism of the anaphylaxis reaction in the rabbit. *J. Immunol.*, 1919, iv, 219.

17. Klinge, F. Die Merkmale der hyperergischen Entzündung. *Klin. Wchnschr.*, 1927, vi, 2265.

18. Rössle, R. Ueber die Merkmale der Entzündung in allergischen Organismus. *Verhandl. d. deutsch. path. Gesellsch.*, 1914, xvii, 281.

19. Auer, J. Local autoinoculation of the sensitized organism with foreign protein as a cause of abnormal reactions. *J. Exper. Med.*, 1920, xxxii, 427.

20. Tannenberg, J. Entzündungsversuche im anaphylaktischen Schock. *Verhandl. d. deutsch. path. Gesellsch.*, 1926, xxi, 144.

21. Nicolle, M. Contribution à l'étude du "phénomène d'Arthus." *Ann. de l'Inst. Pasteur*, 1907, xxi, 128.

22. Opie, E. L. Desensitization to local action of antigen (Arthus phenomenon). *J. Immunol.*, 1924, ix, 247. Acute inflammation caused by antibody in an animal previously treated with antigen; the relation of antigen to antibody in the Arthus phenomenon. *J. Immunol.*, 1924, ix, 255.

23. Grineff, D. L'anaphylaxie locale est-elle justiciable du procédé des petites doses de Besredka. *Compt. rend. Soc. de biol.*, 1912, lxxii, 974.

24. Wells, H. G. The present status of the problems of anaphylaxis. *Physiol. Rev.*, 1921, i, 44.

25. Opie, E. L. The fate of antigen (protein) in an animal immunized against it. *J. Exper. Med.*, 1924, xxxix, 659.

THE EFFECT OF COMBINED POTASSIUM IODIDE AND THYROID SUBSTANCE UPON THE THYROID GLAND *

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In studies made of the effect of potassium iodide and thyroid substance upon the normal thyroid gland of the guinea pig it has been shown [1, 2] that KI has a marked stimulating effect which is most pronounced after from 15 to 20 daily doses of 0.1, 0.05, or 0.01 gm., whereas thyroid substance has a definite depressing effect. In this paper we wish to report our findings concerning the effects of feeding thyroid substance alone, the effects of feeding KI and thyroid together to the same guinea pig and also the effects of feeding one of these substances following a course of the other substance. In a few animals some of the previous experiments were repeated so as to have a control check.

The method used has been described previously [1, 2] and need not be mentioned again. The experiments may be grouped as follows: (1) Two untreated guinea pigs; (2) 2 guinea pigs fed with 0.1 gm. KI daily for twenty days; (3) 11 guinea pigs fed with 0.1 gr. of thyroid substance (Armour & Co.) daily, 6 for thirty days, 5 for twenty days; (4) 14 guinea pigs fed with both KI and thyroid substance, 6 for twenty days, 4 for thirty-two days, 4 for thirty-eight days; (5) 4 guinea pigs fed with KI for twenty days and then thyroid substance for twenty-three days; (6) 3 guinea pigs fed with thyroid substance for thirty days followed by KI for twenty days.

Untreated Control Guinea Pigs: The number of mitoses was 348 and 195. This is well within the control figures.[1, 2] The acini were medium-sized, the epithelium medium cuboidal, the colloid hard with occasional vacuolization and few phagocytes.

KI-Fed Guinea Pigs: The mitoses were very numerous, 2520, and 4364. The epithelium was relatively tall, cuboidal and columnar; the colloid was soft and phagocytes were numerous.

Thyroid Substance-Fed Guinea Pigs: In the description by Gray and Loeb [1] of the effect of thyroid substance upon the thyroid gland

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of the guinea pig, they used only four animals in which accurate quantitative estimation of the mitotic activity was made. These experiments were repeated upon a large series. Since the same results were obtained from both the twenty- and thirty-day feedings with thyroid substance, we shall not differentiate between these two groups in our study.

TABLE I

Number of animals	Number of mitoses	Number of animals	Number of mitoses
5	0	1	20
1	16	2	80
1	18	1	166

Average 34

Table I enumerates the number of mitoses found in the entire thyroid gland; it varies between 0 and 166, with an average of 34. This number is somewhat lower than the average of 64 that Gray and Loeb found; it is markedly lower than the average for the normal control.

Structurally, the thyroid gland showed slight but definite changes. The acini were small and medium in size, being within the range of the control, although on the whole, one would say that the acini were smaller than those found in the thyroid glands of the untreated animals. This difference is too slight to deserve much emphasis. The epithelium was a little lower and the colloid a little harder with less vascuolization than was usually seen in control animals. These experiments show therefore that the feeding of thyroid extract to guinea pigs for a period of twenty to thirty days produces the structural signs of inhibition in the activity of the gland.

The Effect of a Simultaneous Feeding of KI and Thyroid Substance: In this series of experiments 0.1 gm. KI and 0.1 gr. thyroid substance were given daily to the same animal. Of these animals 6 were thus fed for twenty days, 4 for thirty-two days and 4 for thirty-eight days. The number of mitoses obtained in each case is listed in Table II.

It will be noted from Table II that after a period of twenty days of a combined thyroid and KI feeding, the number of mitoses in the entire thyroid gland is very much the same as that found in the un-

TABLE II

20 days	32 days	38 days	20 days	32 days	38 days
398	96	0	18	16	28
188	240	0	0	—	—
0	0	0	570	—	—
	Average	196	88	7	

treated control. If the combined feeding is continued, however, for periods of thirty-two and thirty-eight days, there results a marked diminution in the number of mitoses.

After twenty days of combined thyroid and KI feeding the epithelium becomes somewhat taller than the average untreated control. It is definitely taller than that observed in the glands of animals that have been fed exclusively with thyroid and lower than that seen in animals fed with KI alone. The size of the epithelium under these conditions is somewhat midway between that found in the glands of animals fed with thyroid and those fed with KI. The colloid, on the other hand, is very hard and poorly infiltrated with phagocytes; it is very similar to that seen in animals fed with thyroid substance.

Guinea Pigs Fed with KI for 20 Days Followed by Thyroid Substance for 23 Days: In this series 4 guinea pigs were fed first with a daily dose of 0.1 gm. KI for a period of twenty days and then fed 0.1 gr. thyroid substance for a period of twenty-three days. The number of mitoses per gland in individual cases varied as follows: 54, 48, 32 and 0, giving an average of 33 mitoses. This figure is similar to that obtained in the control series that were fed with thyroid (see above). It appears, therefore, that a preliminary administration of KI to the animal does not counteract in any way the subsequent depressing effects of thyroid substance on the mitotic cell activity of the acinar epithelium. In accordance with this view, we also find the microscopic appearance of the gland to resemble very closely the gland of the control thyroid-fed animal in so far as the colloid is concerned. It is very hard and free from phagocytes; the epithelium, however, is somewhat taller than that seen in animals fed with thyroid alone.

Feeding of KI Following a Preliminary Administration of Thyroid Substance: In these experiments 3 guinea pigs were first fed with daily doses of 0.1 gr. thyroid substance for thirty days, followed by

0.1 gm. potassium iodide for twenty days. In these animals the number of mitoses was 2296, 788 and 656, respectively, giving an average number of 1246 mitoses per entire gland. This figure, although considerably lower than that found in the series that were fed with KI alone, is nevertheless much greater than the average number in the normal controls (see above). Furthermore, the microscopic appearance of these glands is very similar to that noted in the control KI-fed animals; the colloid is softened and very rich in phagocytes, while the acinar epithelium is proportionately increased in size. It follows therefore that a previous administration of thyroid substance to the animals hinders only very little the subsequent effects of KI in so far as structural or proliferative changes in the gland are concerned.

Discussion

The results obtained in these experiments confirm the previous findings as to the manner of the action of potassium iodide and thyroid substance on the activity of the thyroid gland of the guinea pig.

Table III

Normal control	KI feeding control	Thyroid feeding control	KI plus thyroid 20 days	KI plus thyroid 32 days	KI plus thyroid 38 days	KI 20 days thyroid 23 days	Thyroid 30 days KI 20 days
271	3442	34	196	88	7	33	1246

Table III summarizes the average number of mitoses found in the thyroid gland under the different experimental conditions. It shows that KI feeding to normal guinea pigs causes increased cell division, whereas thyroid feeding produces exactly the opposite effect, namely a diminution in cell proliferation. With the combined feeding there was no balance between these two substances, for in twenty days the number of mitoses is not midway between the figures obtained for the KI animals and the thyroid animals, but is very nearly that obtained with thyroid feeding; yet there may be a slight neutralizing effect noticeable. In the thirty and thirty-eight days the thyroid effect is complete; however, in this connection we must recall the fact that a diminished number of mitoses has been found

in KI feeding after twenty-five to thirty days. The other structural changes noted in the gland are essentially those of thyroid feeding.

There are definite average differences in the size of the epithelium between the normal controls, the KI-fed and the thyroid-fed guinea pigs. In the animals which received combined feeding of KI and thyroid, the epithelium is a little taller than in the average control; although the gland in this case is principally under the influence of the thyroid substance, still there is some evidence of KI stimulation. A similar sign of KI stimulation is noted in the fact that in the gland of the animals fed with thyroid for twenty-three days following a preliminary twenty days KI feeding, the epithelium is taller than found in twenty-day thyroid feeding. The picture here is similar to what we have noted when feeding small doses of KI to normal guinea pigs.[3]

The colloid in the animals which received combined feeding, even in the early period of twenty days, is hard and resembles the colloid in the controls fed with thyroid. From the numerous experiments that have been carried out in our laboratory, it is certain that KI has a stimulating effect upon the epithelium of the thyroid gland which is still noticeable when this substance is antagonized by administration of thyroid substance. As far as the colloid is concerned KI and thyroid substance also act antagonistically, the former substance tending to soften it through a direct action of the phagocytes, which are increased in number, upon the colloid. Thyroid substance, on the other hand, causes a hardening of the colloid as well as a diminution in the number of phagocytes.

In the glands of animals fed with KI following thyroid substance the character of the epithelium and of the colloid is similar to that observed in the glands of animals fed with KI that have not received a preliminary treatment with thyroid substance, the number of mitoses seems, however, to be somewhat diminished in these cases. We find thus that the interaction between thyroid substance and KI is similar to the interaction between KI and anterior pituitary substance (Armour & Co.) when fed by mouth, which McCordock[4] has recently described. McCordock finds that through combined feeding of anterior pituitary substance and KI the effect of the latter can be approximately neutralized, although he could also find signs of the effects of the individual substances upon different parts of the gland.

Summary

1. Feeding of potassium iodide to normal guinea pigs stimulates the thyroid gland, whereas feeding of thyroid substance produces the opposite effect.

2. In feeding KI and thyroid concomitantly, the effect of KI is evidenced by the increased size of the epithelium, and the effect of thyroid substance is manifested by the hardness of the colloid and the diminished number of mitoses.

3. Preliminary feeding of KI interferes only slightly with the subsequent depressive action of thyroid substance and similarly a preliminary feeding of thyroid substance affects only to a small extent the subsequent stimulative effects produced by KI feeding.

REFERENCES

1. Gray, S. H., and Loeb, Leo. *Am. J. Path.*, 1928, iv, 257.
2. Rabinovitch, J. *Am. J. Path.*, 1928, iv, 601.
3. Gray, S. H., and Rabinovitch, J. *Proc. Soc. Exper. Biol. & Med.*, 1929, xxvi, 468.
4. McCordock, H. A. *Am. J. Path.*, 1929, v, 171.

PATHOLOGIC HISTOLOGY OF ADRENALECTOMIZED CATS*

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The correlation of the changes occurring during Addison's disease with those of experimental adrenal insufficiency has progressed slowly. In most of the experimental work the animals have survived for too brief a period for easily recognizable pathologic changes to occur. Conversely, in human cases, adrenal insufficiency has been complicated by other factors. Complications may also occur in experimental adrenal insufficiency as noted in this paper and also by Zwemer and Lyons.[1] As shown by an analysis of our cases of uncomplicated adrenal insufficiency, the histologic changes are roughly proportional to the length of survival after adrenal removal, and were first distinguished in our longest surviving animals.

The experimental work included the gross and microscopic examination of the tissues obtained from ten cats that had had both adrenals removed. The survival period following adrenalectomy averaged 240 hours, and varied between about 100 and 300 hours. No effort was made to prolong the life of these animals by administration of solutions or extracts. However, we exercised particular preoperative and postoperative care and strict asepsis during operations. As controls we used five normal, healthy cats, two cats that were kept continually in deep anesthesia with ether for ten successive hours, two cats that were starved, and two cats that died from bilateral confluent pneumonia.

To obtain fresh tissues, the animals were killed when they appeared extremely feeble. Complete autopsies were performed including the brain and upper portion of the spinal cord. Tissue was cut in small pieces 2 to 3 mm. in thickness, fixed in Zenker's solution,

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formalin, and alcohol-formalin, embedded in celloidin and paraffin, and stained with hematoxylin and eosin, and eosin and methylene blue. Frozen sections of formalin-fixed tissue were stained with Sudan IV, Nile blue sulfate, and osmic acid.

Cultures were taken at the time of the autopsy from the peritoneal cavity and heart's blood and planted in plain broth, and milk, and also on sheep blood agar plates. In this series of ten test animals we have included only those in which the cultures were sterile. Nearly as many animals in which we obtained positive cultures had to be discarded. The survival period of the infected animals was much shorter than that of the uninfected group. This agrees with the known susceptibility of adrenalectomized animals to toxins.

A gross examination of the organs and tissues of the animals suffering from adrenal insufficiency reveals little that is unusual. The amount of subcutaneous fat is diminished, although the fat in the mesentery and great omentum is still quite abundant. The body is dehydrated, the surfaces of the peritoneal cavity though smooth, lack their usual sheen. The liver and spleen are moderately congested, and the blood that flows from the freshly cut surfaces is unusually viscid. The subcutaneous, mesenteric and mediastinal lymph nodes are enlarged, firm and pale pink. The kidneys are large, the capsules strip readily from opaque yellow smooth surfaces. The fresh surface of the kidney is rather startling, the cortex is so opaque, yellow, regular and thickened, in contrast to the pyramids which are moderately congested. The heart, lungs, pancreas, intestines, bladder, skeletal muscle, testis, brain, cord, pituitary, thyroid, parathyroid, aorta and bone marrow show no constant gross lesions.

In résumé, the gross findings are fatty degeneration of the kidney, enlargement of the lymph nodes, dehydration, and moderate congestion of the liver and spleen.

Microscopic Report

The histologic changes which appear to be constantly associated with adrenal insufficiency are confined to the kidney, lymphoid tissue throughout the body, thyroid and testis. The lesions were alike in all cases and varied only in degree, depending largely on the period of survival.

Kidney: The microscopic findings in the kidney are even more

striking than the gross picture. The lesion is confined to the tubules, especially the convoluted portions, leaving the glomerular capsule and capillary tufts unaffected.

The tubular epithelium from the kidney of a normal cat contains a moderate amount of fat which varies slightly in different animals. In the adrenalectomized cat the amount of fat in the epithelial cells lining the tubules is greatly increased.

In comparing the kidneys from the normal cats, the cats that had received a large dose of ether, those that died from an acute infection, and the adrenalectomized cats, the contrast between the last named group and the first three is quite remarkable. On the other hand there was no appreciable variation in either the appearance, staining reaction or amount of visible fat in the tubular epithelium of the normal, starved, and etherized cats.

The normal kidney, in the eosin-methylene blue preparations, has convoluted tubules that have very distinct lumina bordered with well defined, rather cuboidal epithelium. The cytoplasm of these cells, which is fairly dense, homogeneous and eosin-staining, contains minute to medium-sized clear vacuoles that are usually situated toward the base of the cell. With the common differential fat stains these vacuoles are shown to represent fat droplets that stain orange with Sudan IV, black with osmic acid and pink with Nile blue sulfate. No visible fat was present in the glomeruli, in the lumina of the tubules, in the epithelium of the collecting tubules, in the stroma or in the blood vessels.

In the kidney of the adrenalectomized cat the cells lining the convoluted tubules are poorly defined, irregular and so distended with large and small vacuoles that they appear to obliterate the lumina. Even the cytoplasm of the cells lining the loops of Henle and the collecting tubules contains large and small clear vacuoles. Sections stained with Sudan IV, Nile blue sulfate, and osmic acid show no fat in the glomeruli. The fat in the convoluted tubules is very abundant and stains orange-red with Sudan IV, black with osmic acid and pink with Nile blue sulfate. In the collecting tubules the fat stains quite differently; with Sudan IV, it appears as fine orange-yellow granules, which fail to stain with osmic acid, and stain dark blue with the Nile blue sulfate. Furthermore, small fatty casts are seen in the lumina of the tubules. These stain orange-red with Sudan IV, and black with osmic acid, whereas with Nile blue sulfate the casts in

the convoluted tubules stain pink and those in the larger collecting tubules stain both pink and blue. This differentiation suggests that the source of the casts is probably in the tubular epithelium; the pink-staining casts arising from degenerating cells of the convoluted tubules and the blue from the more distal collecting tubules.

Small fat droplets were frequently seen in the larger blood vessels. These stained orange with Sudan IV, black with osmic acid, and some blue and some pink with Nile blue sulfate.

In summing up the histologic changes in the kidney, it is clear that the tubules show a definite lesion in which the amount of fat is greatly increased. In the convoluted tubules this stains as neutral fat, while in the collecting tubules it appears as fatty acids. In the collecting tubules fatty casts are seen that probably arise from the lipoid degeneration of the tubular epithelium.

Lymphoid Tissue: In both the spleen and lymph nodes there is marked lymphoid hyperplasia. The lymph nodules are increased in size, the germinal centers are large and filled with young lymphocytes and lymphoblasts in which mitoses are numerous. Mature lymphocytes distend the sinuses and infiltrate the stroma of the lymph nodes.

This finding is entirely different from the observation of Banting and Gairns [2] on dogs, in which the lymph nodes showed enlargement with edema, a tremendous proliferation of plasma cells, endothelial leucocytes, enlarged macrocytes and occasionally polymorphonuclear leucocytes, and also a great increase in the reticular tissue, compression and almost complete obliteration of the germinal centers, active phagocytosis of red and white blood cells, and frequently blood pigment.

Zwemer and Lyons have recently observed that there is an absolute increase in the lymphocytes in the circulating blood of adrenalectomized cats, a finding that may be correlated with the histologic changes in the lymphoid tissue in our series of "test" animals.

Thyroid: In most of the animals and especially in those with longer survival periods, the follicles show a moderate degree of colloid distention. The colloid is homogeneous and stains a pale pink with eosin. The follicular epithelium is low cuboidal or slightly flattened.

Testis: The interstitial cells of the testis are increased in size and number. Their cytoplasm is distended with minute clear vacuoles of

uniform size. They morphologically resemble the cells in the outer layer of the cortex of the suprarenal gland. It is said that the number and size of the interstitial cells vary greatly under normal conditions, hence our observation, which is based on a rather small group of animals, may simply be a chance finding. However, this increase occurred in all of the test animals and appeared to vary with the duration of survival. When stained with Sudan IV, osmic acid or Nile blue sulfate the cells resemble in every way those of the cortex of the normal adrenal gland of the cat.

Discussion

Several points must be carefully considered in interpreting the histologic changes that appear to follow bilateral adrenal removal. First, are the changes merely constitutional variations that might be found in any group of animals, or are the changes constant, and do they vary in degree depending on the period of survival? Secondly, can one be certain that removal of the adrenal glands leaves no accessory adrenal tissue, and what part does infection, ether inhalation, dehydration or thirst play in producing the histologic changes?

In answer to the first question we feel that the histologic findings are quite different in the "control" and "test" animals, and cannot be accounted for simply on the basis of unusual constitutional variations within the normal. Moreover, these changes occurred with definite regularity and in most cases were accentuated in the long-surviving animals.

It is impossible to be absolutely certain that all adrenal tissue has been removed, yet in the cat the presence of accessory adrenal tissue is a very unusual finding, and if present even in small amounts will probably maintain the life of the animal. An example from one of our own series will make this point clear. A large cat that made a good recovery after bilateral adrenalectomy, began on the second day after the operation to show signs of adrenal insufficiency which slowly progressed for about eight days. The animal then remained in a lethargic semicomatose state for several days when definite signs of improvement were noticed. The animal gained in weight and showed normal behavior and lived as a normal appearing animal for sixty-seven days. At this time an exploratory operation was done in search of accessory adrenal tissue. A clean dissection was

made of the old adrenal beds and of loose tissue lying adjacent to the large vessels. Multiple sections were taken from this and revealed in addition to scar tissue, lymph nodes and sympathetic ganglia, a small encapsulated nodule of large adrenal cortical cells that were finely vacuolated. The animal lived thirteen days longer showing progressive signs of adrenal insufficiency until death.

Every effort was made during the operations and in the after care of the animals to prevent infection. Cultures, taken from the heart's blood and peritoneum of the animals that we have included in the normal control and operated series, were sterile.

Prolonged ether inhalation, starvation or dehydration, produced no constant lesions that would be confused with the histologic lesions we have described.

Another question that at once suggests itself is what relationship does adrenal insufficiency bear to the histologic lesions? Are these lesions dependent primarily on lack of adrenal tissue and more particularly the cortical portion, or are they secondary to metabolic or biochemical changes resulting from adrenalectomy? On the other hand, may not the chemical changes be simply the result of degenerative changes in the kidney, thyroid and other organs? Such questions as these cannot be answered from a purely histologic study but there are several points that may have a clinical bearing and warrant further consideration.

The problem of "lipoid nephrosis" is one that is receiving a great deal of attention at the present time from both clinicians and pathologists. If we restrict ourselves to the pathologic interpretation of the term whereby we imply simply fatty degeneration of the tubules of the kidney, as was done by Hartman and his collaborators,[3,4] then we may say that adrenalectomy is at least one means of producing this pathologic entity. However, the term "lipoid nephrosis" is in reality a name applied to a clinical syndrome of which a fatty degenerative change in the tubules of the kidney is but one, and probably, a relatively late manifestation.

The characteristics of "lipoid nephrosis" as reported by Epstein [5] are edema, albuminuria, low basal metabolism and changes in the composition of the blood, with absence of hypertension, retinitis, hematuria and azotemia. The changes in the blood are a decrease in the total serum protein, with an inversion of the normal albumin-globulin ratio, and in most cases a decrease in the blood calcium.

No edema is present in animals suffering from adrenal insufficiency. On the contrary, the tissues appear dehydrated (Zwemer [6]). The lowered blood pressure (Marshall and Davis [7]) and fall in basal metabolism (Aub, Forman and Bright, [8] Aub, Bright and Forman, [9] Zwemer and Bright [10]) are similar in both conditions. There are increases in total blood solids (Donath, [11] Hartman et al., [3] Lucas [12]) and in serum calcium (Kisch, [13] Hartman, [3] Stewart and Rogoff [14]) after adrenal removal, in contrast to the "lipoid nephrosis" observations.

Although there are not many points in common with adrenal insufficiency, the changes in blood pressure, basal metabolism, and the kidneys suggest a possible endocrine disturbance present in the clinical syndrome known as "lipoid nephrosis."

Perhaps the most interesting lesion was the marked hyperplasia of the lymphoid tissue. Several years ago the clinical relationship of status lymphaticus and adrenal insufficiency was described (Hedinger,[15] Wiesel,[16] Fahr and Reiche [17]). It would appear that there might be experimental evidence to substantiate that observation, and further it suggests that the lymphoid tissue, whatever its function is, may be physiologically related to the glands of internal secretion. On the other hand the hyperplasia may be only the response to an unusual toxemia. The histologic picture of the lymph nodes substantiates the lymphocytosis previously described by Zwemer and Lyons.

In a few cases of Addison's disease in which the testis was studied microscopically, unusually large and numerous interstitial cells were observed. These and our experimental observations indicate a possible adrenal-interstitial relationship, which is strengthened when we remember their common embryological origin, and the data collected by Glynn [18] on the relation of adrenal hypertrophy to clinical sex anomalies.

It is exceedingly difficult, if not impossible to determine the physiologic activity of a tissue by examination of fixed and stained sections thereof, but, the thyroid glands in our operated animals appear to be in what is frequently called the "resting state." If this is true, it may account in part for the decrease in basal metabolism after adrenalectomy and add something to the interpretation of thyroid-adrenal activity.

SUMMARY

1. The gross and miscroscopic lesions following experimental adrenal insufficiency in cats (average survival ten days) are described.

2. Briefly they consist in fatty degeneration of the tubules of the kidney, lymphoid hyperplasia, moderate colloid distention of the thyroid, and hyperplasia of the interstitial cells of the testis.

3. The significance of these, and other observations on experimental adrenal insufficiency, is discussed in relation to "lipoid nephrosis," lymphoid hyperplasia and additional clinical syndromes.

NOTE: We are indebted to Dr. F. B. Mallory for the photomicrographs and to Miss Marion E. Lamb for technical assistance.

REFERENCES

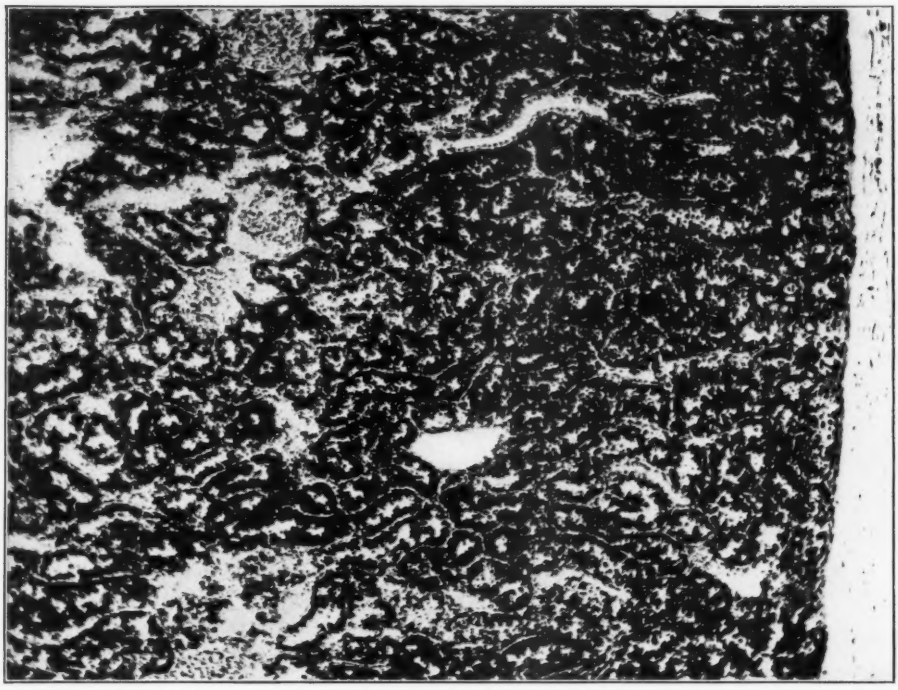
1. Zwemer, R. L., and Lyons, C. *Am. J. Physiol.*, 1928, lxxxvi, 545.
2. Banting, F. G., and Gairns, S. *Am. J. Physiol.*, 1926, lxxvii, 100.
3. Hartman, F. A., MacArthur, C. G., Gunn, F. D., Hartman, W. E., and MacDonald, J. J. *Am. J. Physiol.*, 1927, lxxxi, 244.
4. Gunn, F. D., Cori, C. F., and Hartman, F. A. *Proc. Soc. Exper. Biol. & Med.*, 1928, xxv, 410.
5. Epstein, A. A. *Arch. f. Verdauungskr.*, 1928, xliv, 31.
6. Zwemer, R. L. *Am. J. Physiol.*, 1927, lxxix, 658.
7. Marshall, E. K., Jr., and Davis, D. M. *J. Pharmacol. & Exper. Therap.*, 1916, viii, 525.
8. Aub, J. C., Forman, J., and Bright, E. *Am. J. Physiol.*, 1922, lxi, 326.
9. Aub, J. C., Bright, E., and Forman, J. *Am. J. Physiol.*, 1922, lxi, 349.
10. Zwemer, R. L., and Bright, E. (In Press.)
11. Donath, J. *Arch. f. exper. Path. u. Pharmakol.*, 1914, lxxvii, 1.
12. Lucas, G. H. W. *Am. J. Physiol.*, 1926, lxxvii, 114.
13. Kisch, B. *Klin. Wchnschr.*, 1924, iii, 1661.
14. Stewart, G. N., and Rogoff, J. M. *Am. J. Physiol.*, 1928, lxxxvi, 25.
15. Hedinger, E. *Verhandl. d. deutsch. path. Gesellsch.*, 1907, xi, 29.
16. Wiesel, J. In: Handbuch der Neurologie; von M. Lewandowski, iv. J. Springer, Berlin, 1913.
17. Fahr, T., and Reiche, F. *Frankfurt. Ztschr. f. Path.*, 1919, xxii, 231.
18. Glynn, E. E. *Quart. J. Med.*, 1912, v, 157.

DESCRIPTION OF PLATES

PLATE 99

FIG. 1. Photomicrograph of kidney stained with Sudan IV, showing the large amount of fat in the tubular epithelium. × 100

FIG. 2. Photomicrograph of portion of lymph node, showing hyperplastic lymph nodules and the peripheral sinus distended with mature lymphocytes. × 150



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MacMahon and Zwemer Pathologic Histology of Adrenalectomized Cats

CONGENITAL ANOMALIES OF THE LIVER *

H. E. MacMahon, M.D.

(From the Pathological Laboratory of the Boston City Hospital, Boston, Mass.)

The microscopic study of a large series of livers from premature and full-term infants, infants one to several months of age, children and adults has shown clearly that congenital anomalies may occur within the liver in regard to the arrangement of the liver cells, the lobules, the intrahepatic bile ducts, and also in the mesenchymal architecture of the portal areas. The importance of these variations is probably more of embryologic or biologic interest, and yet they should be recognized by the pathologist as forming a histologic basis of some of the more unusual forms of intractable jaundice of infancy. Furthermore, they should be considered carefully both in the differential diagnosis of healed forms of cirrhosis and in the interpretation of some of the unusual histologic lesions at times seen in the fully developed liver.

Embryology of the Liver

The development of the human liver, according to the majority of anatomists, recapitulates broadly the forms recognized in the evolution of the animal scale. The liver begins as a ventral outgrowth of the entodermal tube, a stage seen in the amphioxus in which the liver is merely a cecal diverticulum of the digestive canal. In the shark, this outpouching has developed into considerable size; it is formed of epithelial tubes with lumina, known as hepatic cylinders. In the amphibians there is further development, the cylinder and lumina are smaller, and the organ is a modified tubular gland composed of these cylinders of hepatic cells arranged around bile ducts. A similar picture is seen in the 10, 12, 14 and 16 mm. human embryo, in which the liver is composed of a network of reticulating cylinders bordered by endothelial-lined spaces, but at this stage the intrahepatic bile ducts have not yet developed.

In the further development of the embryo the tubular form is lost and smaller and smaller meshes of hepatic cells arise from these

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primary cylinders. At first these appear simply as a network of trabeculae lacking an orderly arrangement, but later they become grouped into lobular units.

There are different theories regarding the embryogenesis of the intrahepatic bile ducts. Minot [1] favored the view that they arose from the hepatic duct — "the origin of the interlobular ducts is as yet but imperfectly understood, but we may surmise that they arise as evaginations of the hepatic duct: certain it is that they are always distinct from the hepatic cells." According to Arey,[2] "at eight weeks hollow interlobular ducts appear spreading inward from the hepatic duct along the larger branches of the portal vein." Schäfer,[3] Lewis,[4] Hertwig,[5] von Meyenberg [6] and Keith [7] regard at least the smaller periportal ducts as arising from the liver cells, while von Meyenberg in his interpretation of liver cysts concluded that the large periportal ducts arose from the hepatic duct. According to Keith, "the ducts of the liver, unlike those of any other gland, arise by a secondary process. Undifferentiated cells lying along the distribution of the portal vein in the liver, group themselves into cords, develop lumina, become covered with mesodermal tissue and then form the intrahepatic bile ducts."

Lewis describes the process as follows: "The trabeculae form cords extending along the surface of the periportal mesenchyma and in them a lumen is formed. In places the cells on the mesenchymal side of the lumen are distinctly flatter than those toward the portal capillaries. In a later stage the mesenchyma has increased so that it surrounds the ducts which were seen forming along its surface. Their epithelium has become regularly cuboidal or columnar."

The commonly accepted view regarding the development of the extrahepatic biliary system is that the hepatic ducts arise within the stalks of the solid hepatic buds, that is from the anterior portion of the hepatic diverticulum; while from the caudal portion are developed the common bile duct, the gall bladder, and the cystic duct at the junction of the common bile duct and the gall bladder. Moreover, in addition to arising from different portions of the original hepatic anlage, these structures develop in different stages so that one portion may have a lumen while another is still a solid cord of cells.

The primary vessels of the liver are the two vitelline veins which form a close anastomosis with each other. With invasion by liver

tissue and the formation of sinusoids, these veins become resolved into a distributing and collecting group. The distal group comprises the system of hepatic veins, whereas the proximal portion of the two vitelline veins forms a single vessel which represents the portal vein of later stages. About the fifth week of embryonic life the left umbilical vein establishes a communication with the vascular plexus of the liver, so that placental blood as well as blood from the intestines now passes through the liver. Soon afterward, a large channel, the ductus venosus, develops between the proximal and distal groups of vitelline vessels; this conveys the larger portion of placental blood directly into the inferior vena cava.

Very early the common bile duct, the cystic and hepatic ducts are surrounded by mesenchyma. With the development of the hepatic circulation the mesenchyma spreads along the ramification of the portal vein into the substance of the liver. Periportal ducts form, and branches of the hepatic artery, together with nerves and lymphatics extend along into this loose mesenchyma so that the wide portal areas show a rather sharp contrast to the little connective tissue that surrounds the thin-walled central veins.

In brief, the development of the mammalian liver may be traced from a simple diverticulum of the foregut, through a compound tubular structure composed of reticulating cylinders, through a stage in which the liver is composed of a network of liver cell trabeculae lacking orderly arrangement, and finally into the mature form in which it is divided into lobular units. The peripheral bile ducts appear to develop from undifferentiated parenchyma cells bordering the portal areas. These form larger ducts which communicate with branches of the hepatic duct which arise outside of the liver. The gall bladder, cystic and common ducts develop independently. The mesenchyma of the portal areas extends in along the portal vein and increases with the appearance of branches of the hepatic artery, nerves and lymphatics.

In this short review of the embryology of the liver we have stressed a few of the more important developmental processes which form the basis of some of the more common congenital anomalies seen in later months.

GROSS ANOMALIES

The number of anomalies that might arise in an organ having such a complicated process of development could be very large, and probably many of the minor variations are often overlooked.

Holmes [8] describes congenital obliteration of the bile ducts as they affect the hepatic, cystic, common duct and gall bladder, and reviews the gross morphologic relation in one hundred reported cases. He favors the view that the obliteration is a developmental anomaly rather than infectious, and points out that in at least sixteen of these cases surgical relief was possible, while Thomson[9] first drew attention to the cirrhosis of the liver which may result from an obliterative process in the large biliary system.

Boyden [10] has described the gross morphologic anomalies of the mammalian gall bladder. Meckel [11] reported a case of complete absence of the gall bladder, and Kermisson and Hébert [12] collected three cases in which there was no trace of the hepatic, cystic or common ducts.

In the liver, the more common gross anomalies are irregularities in form, in the number of lobules and in the presence of cysts; while a very much rarer finding is the presence of one or more accessory livers.

HISTOLOGIC ANOMALIES

The congenital histologic variations of the liver, exclusive of those changes which occur as a result of some abnormality in the extrahepatic biliary system, may be separated into two groups: first, those in which there has been simply a retardation in the normal process of development, in which case the liver of a full-term child may structurally resemble, in part at least, the liver of a developing fetus; and secondly, those in which there is a true developmental anomaly involving either the parenchyma or the connective tissue forming the portal areas.

GROUP I. Two varieties were found that fall into this group. In one which occurred in a full-term child who lived seven months the structure resembled a network of reticulating cylinders, in which tubules of eight and ten liver cells surround large and small lumina. This simulated in arrangement the developing liver of a young mammalian embryo, or the mature liver of an amphibian.

The second example occurred in a full-term child who lived several months and was jaundiced since birth. As in the former case, the extrahepatic biliary system was normal. The liver was composed of an anastomosing network of poorly defined trabeculae of liver cells separated by wide endothelial-lined spaces. The liver cells varied in size and shape and were poorly defined, while some contained six and eight nuclei. They were arranged in single rows, sometimes in double rows with a distended bile capillary between. There was no lobulation, but here and there the parenchyma was traversed by irregular portal areas in which normal periportal ducts could be followed. The jaundice which occurred in this case probably resulted from a lack of continuity of the bile capillaries, which in turn was dependent on the disorderly arrangement of the liver cells.

GROUP II. Probably one of the commonest variations seen in the liver of newborn infants is the irregularity in the amount of mesenchyma in the portal areas. Sometimes an unusually large amount of connective tissue has grown in along the large vessels. In the infant this tissue may be arranged loosely and appears very conspicuous. In later life with condensation and increase in the number and size of the lobules it becomes a much less noticeable feature. At either age, it appears to cause no functional disturbance. Two of the cases of this type were complicated by the presence in the portal connective tissue of many blood-forming cells similar to those lying in the perisinusoidal spaces.

Another type of anomaly likewise involving primarily the mesenchyma occurred in a full-term child who lived nine weeks. This child had also been jaundiced since birth. The portal connective tissue in all portal areas was increased in amount and was very condensed. The portal vessels were numerous, small and compressed. The periportal ducts were increased in number and could be traced as double rows of cells often showing no lumen. These duct cells resembled the poorly differentiated embryonic type, and lacked the normal development into the mature cuboidal and columnar forms. It is possible that this abnormality is primarily a maldevelopment of the periportal ducts; however, the increase and condensation of the mesenchyma is such an outstanding feature that the duct anomaly is probably secondary.

The normal liver of a pig and also of a camel (Mall [13]) is divided into distinct and separate lobules by strands of connective tissue.

Occasionally the human liver may show this reversion in varying degrees. The human liver may be resolved into well defined lobules partly by rows of liver cells at the periphery which lie at right angles to the radiating trabeculae, and partly by narrow bands of connective tissue.

This division into lobules may be much more irregular and much more complete. Narrow bands of mesenchymal tissue may extend from one portal area to another dividing the parenchyma into single and compound lobules, often lacking any visible central veins. In one case of this type, in a child twenty months old showing other congenital stigmata, the peripheral connective tissue was bordered by young sprouting periportal ducts which had not become detached from the lobule by the surrounding mesenchymal tissue (Figs. 1 and 2).

This last case is of unusual interest because it shows a complete border of periportal ducts developing from the undifferentiated peripheral parenchyma cells. Bloom [14] has recently attempted to prove that the change of liver cell cords into bile duct epithelium is dependent on the former being surrounded by connective tissue. According to this author, "when the connective tissue is absent, the epithelium surrounding the lumen of the tube is continuous and morphologically indistinguishable from the remainder of the hepatic parenchyma." In the case reported here this transformation occurs in the absence of surrounding connective tissue, which suggests that some factor other than connective tissue may control the embryogenesis of these periportal ducts. Furthermore, there is evidence to show that the cells of the periportal ducts are relatively less differentiated in the infant than in the adult. In an adult, a toxin that kills off all the liver cells of a lobule leaving only the periportal ducts, results in the regeneration of cells, simulating the periportal duct cell, which apparently never form true liver cells. In an infant's liver that has suffered a similar destructive lesion, the periportal cells may regenerate in the form of tubules, resembling by the morphology of the cell and the presence of bile in the lumina, liver cells; and by the tubular arrangement of the cells, poorly formed bile ducts (Fig. 3).

There is another anomaly of the liver which occurs more frequently that is characterized by an increase in number and size of the periportal bile ducts. Many of these, even in the seven-month

fetus, are unusually dilated and appear as distended tubules and small irregular cysts. These cyst-like ducts contain eosin-staining finely granular coagulum. The cells lining the dilated ducts are small, rather uniform and cuboidal, and have little cytoplasm, whereas the nucleus is relatively large and resembles that in the liver cells. At the margin of the portal areas are ducts that are incompletely formed; the row of cells lying adjacent to peripheral mesenchyma resembles bile duct epithelium, whereas the cells toward the lobule are like liver cells and are continuous with the liver cells of the lobule.

A well marked case in an eight-month fetus showed the liver parenchyma divided up irregularly into uneven lobules by coarse bands of loose mesenchyma. The large, dilated, poorly formed periportal bile ducts were distributed along the surface of the portal connective tissue (Fig. 4). The larger portal areas contained as many as six to eight thin-walled moderately large blood vessels.

In another case, in a full-term child one month old, the many dilated ducts were less prominent. The cells lining the ducts were uniform in size and better defined, but were still relatively undifferentiated. There was more connective tissue in the former case, and most of the ducts were completely surrounded by it.

The liver of a child three months old showed a similar picture. The number of dilated bile ducts was about the same as that in the earlier cases, but with further condensation of the stroma they were less conspicuous. They did not appear to be increasing in number or in size, and the duct cells showed no mitoses. The cells were more mature and in places tended to become low columnar, with the nuclei more toward the base. Normal portal areas were seen at the margin of many of the more recently formed lobules, but the greater part of the liver still lacked normal lobulation (Fig. 5).

This type of anomaly persists with the growth of the individual. An excellent example was seen in a child 15 years of age. The large portal areas showed an increase in both number and size of the ducts as well as an increase in blood vessels and connective tissue. The epithelium of the ducts was generally of a very uniform cuboidal type, although at times it was low columnar. The presence of bile within these ducts demonstrates that they are part of the functioning biliary system (Fig. 6).

The liver of an adult, which grossly may appear quite normal, may

show this same malformation. Here, however, with the growth of liver cells and condensation of the portal connective tissue, this anomaly which formed such an outstanding feature in the fetus may appear relatively insignificant (Fig. 7).

Sometimes, as not infrequently happens both in the embryo and adult, a portion of a duct becomes detached and separated off as an epithelial-lined cyst. This may increase in size and result in compression of the surrounding stroma. The watery fluid that is secreted into the cyst may so distend the wall that with gradual thinning and elongation of the epithelial cells they resemble a delicate wall of endothelium (Fig. 8). In time the epithelium may atrophy, and then the fluid is absorbed, the walls contract, and the space becomes obliterated by fibrous tissue.

In this type of congenital malformation of the ducts the process begins with an abnormal development of the periportal bile ducts. When once formed they do not disappear but become gradually less conspicuous with the later development of the liver. Whether the anomaly is primarily the result of anomalous development of the periportal bile ducts, or whether it is secondary to an increase in mesenchyma that is commonly present, is a debatable question; however, the unusual regularity with which this anomaly is associated with the bilateral congenital cystic kidney would suggest that we are dealing with a primary developmental defect of parenchymatous origin. In the infant the liver may be normal in size, contour and color. It may cut with slightly increased resistance. Fibrous bands may be irregularly followed extending into the liver from the transverse septum. In the adult the liver may be grossly normal or filled with large and small cysts. According to Moschcowitz [15] the congenital cysts of the liver arise from aberrant ducts, one type of cyst arising from "inflammatory hyperplasia of these ducts," the other by retention of fluid as a result of congenital obstruction.

SUMMARY

Congenital anomalies of the liver are relatively infrequent. They may involve the biliary system, the liver cells or the stroma. Several of the more common types are described and explained on an embryologic basis. One type that is characterized by an increase and dilatation of the periportal bile ducts, accompanied by an in-

crease in connective tissue in the portal areas, may persist throughout life. Associated with this type of anomaly are the congenital cysts of the liver occasionally seen at birth and also in later life. Moreover, this anomaly, with or without cyst formation, very commonly accompanies bilateral congenital cysts of the kidneys.

REFERENCES

1. Minot, C. S. Human Embryology. New York, 1892.
2. Arey, L. B. Developmental Anatomy. Philadelphia, 1924.
3. Schäfer, E. A. Quain's Elements of Anatomy, i. London, 1898.
4. Lewis, F. T. Keibel and Mall, Manual of Human Embryology, ii. Philadelphia, 1912.
5. Hertwig, O. Lehrbuch der Entwicklungsgeschichte des Menschen und der Wirbelthiere, Ed. 10. Jena, 1915.
6. von Meyenberg, H. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1918, lxiv, 477.
7. Keith, Sir A. Human Embryology and Morphology. London, 1923.
8. Holmes, J. B. *Am. J. Dis. Child.*, 1916, xi, 405.
9. Thomson, J. *Edinburgh M. J.*, 1892, xxxvii, 604.
10. Boyden, E. A. *Am. J. Anat.*, 1926–27, xxxviii, 177.
11. Meckel, J. F. Handbuch der pathologischen Anatomie. Leipzig, 1812.
12. Kirmisson and Hébert. *Bull. et mém. de la Soc. anat. de Paris*, 1903, lxxviii, 317.
13. Mall, F. B. *Am. J. Anat.*, 1906, v, 227.
14. Bloom, W. *Am. J. Anat.*, 1926, xxxvi, 451.
15. Moschcowitz, E. *Am. J. M. Sc.*, 1906, cxxxi, 674.

DESCRIPTION OF PLATES

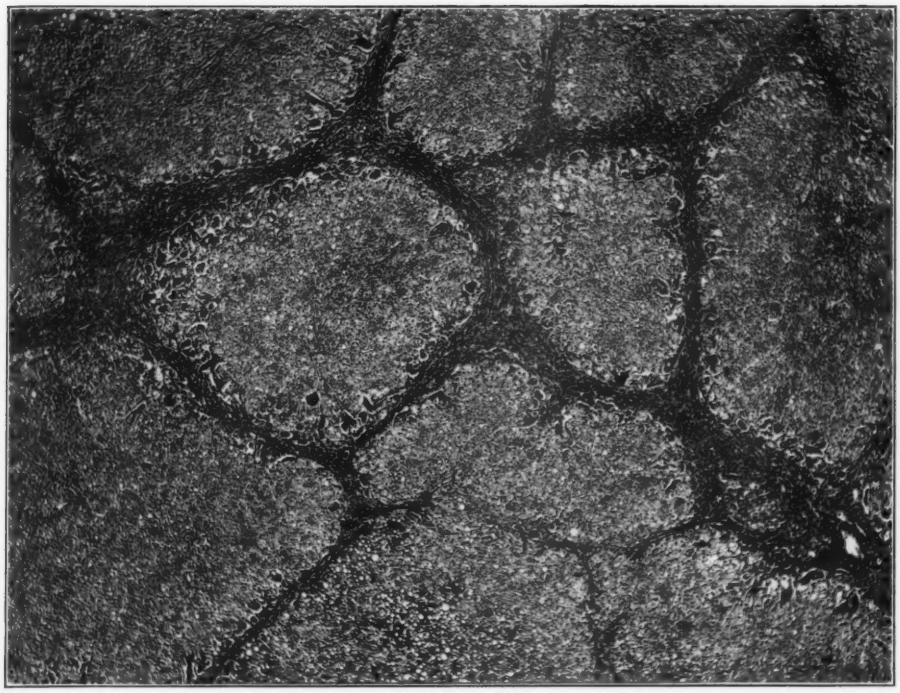
PLATE 100

FIG. 1. Photomicrograph of the liver of a full-term child who lived twenty months. The parenchyma is divided into single and compound lobules, many of which lack central veins. Developing periportal bile ducts border the periphery of the lobules. × 50.

FIG. 2. A higher power photomicrograph of the liver in Fig. 1. The portal connective tissue contains well formed vessels and bile ducts. The small embryonic periportal bile ducts lie at the periphery of the lobule. The liver cells lack uniformity. The trabecular arrangement is lost, and bile capillaries are indistinct. × 150.

XENOS

XI



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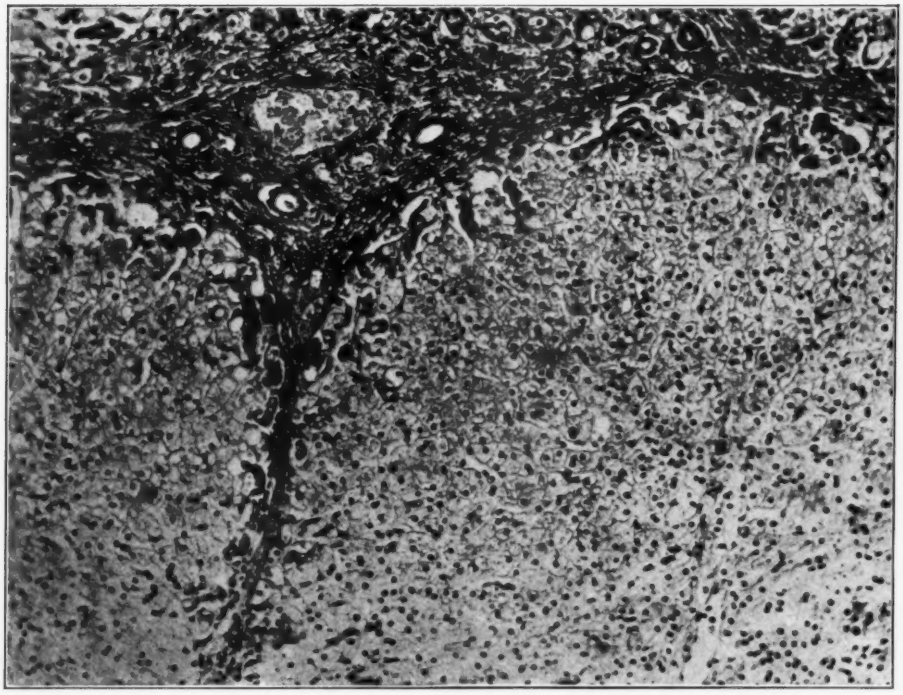

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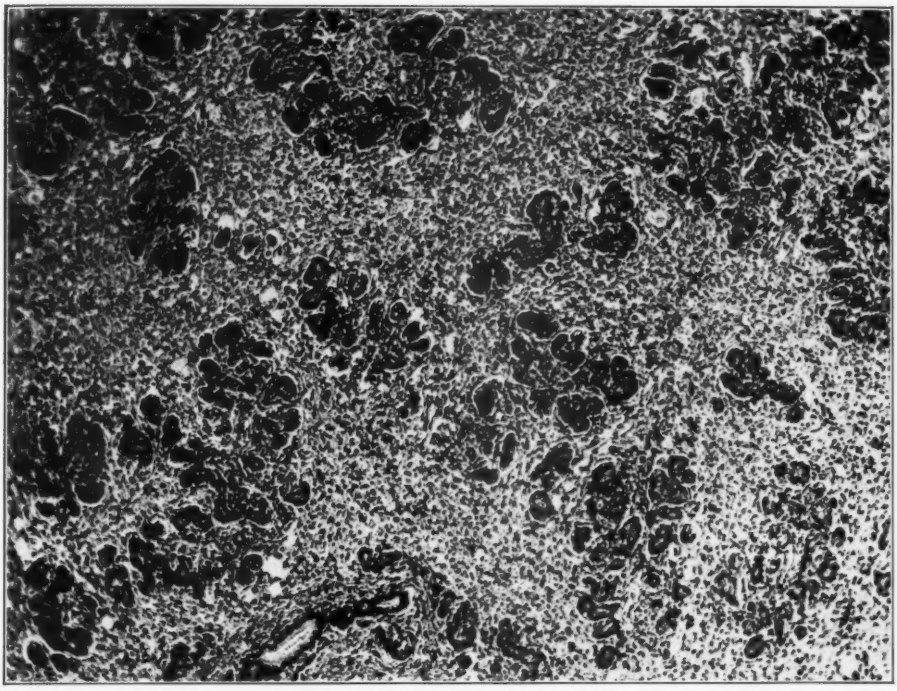
PLATE 101

FIG. 3. Photomicrograph of the liver of a three-months-old child showing the healing stage of an acute toxic hepatitis. The liver cells of the lobules have been killed. The trabecular spaces are filled with endothelial leucocytes. Regenerating periportal bile ducts border the portal areas. The morphology and the presence of bile in bile capillaries bordered by the cells suggest that they are liver cells, whereas their arrangement more closely resembles poorly formed bile ducts. × 100.

FIG. 4. Photomicrograph of the liver of an eight-month fetus. Large dilated cyst-like periportal ducts border the peripheral mesenchyma. The liver cells are separated by wide endothelial-lined sinusoids and are arranged as a loose network of interlacing trabeculae. There are many foci of cells showing hematopoiesis in the perisinusoidal spaces. × 100.



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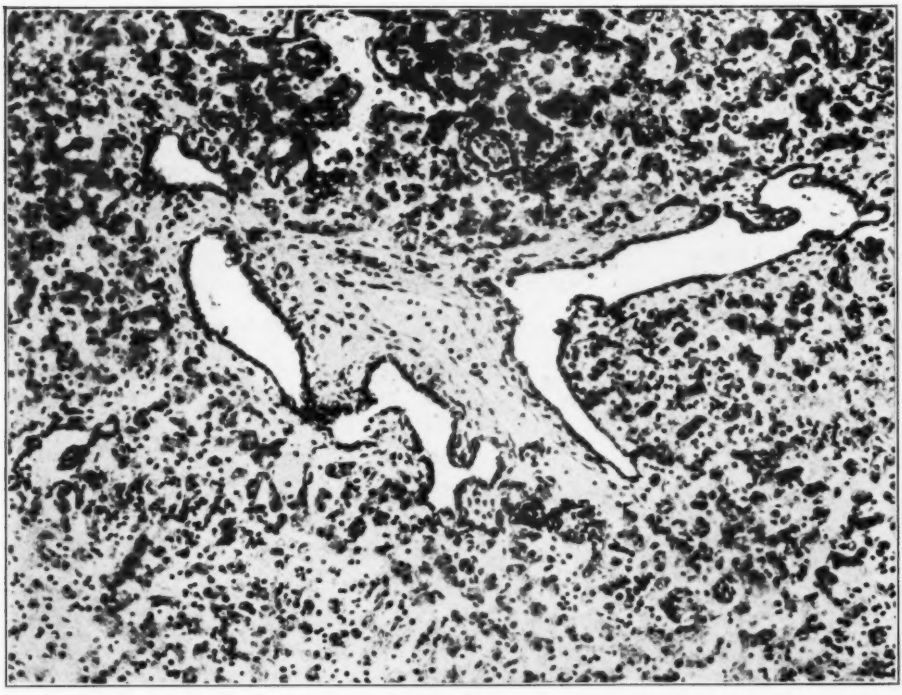

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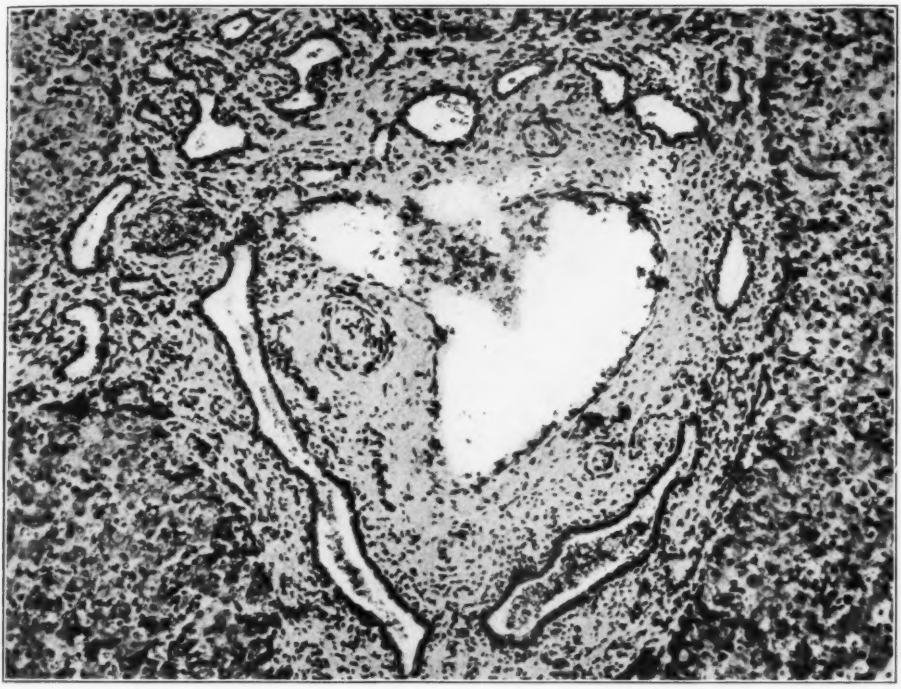
PLATE 102

FIG. 5. Photomicrograph of the liver of a full-term child who lived for three months. The many dilated bile ducts stand out in sharp contrast to the moderately condensed stroma. The cells lining the bile ducts are uniform in size and more differentiated than in the preceding case. × 150.

FIG. 6. Low power photomicrograph of the liver of a child 15 years of age. The larger portal areas contain many dilated bile ducts. The presence of bile in some of these suggests that they form part of the functioning biliary system. × 50.



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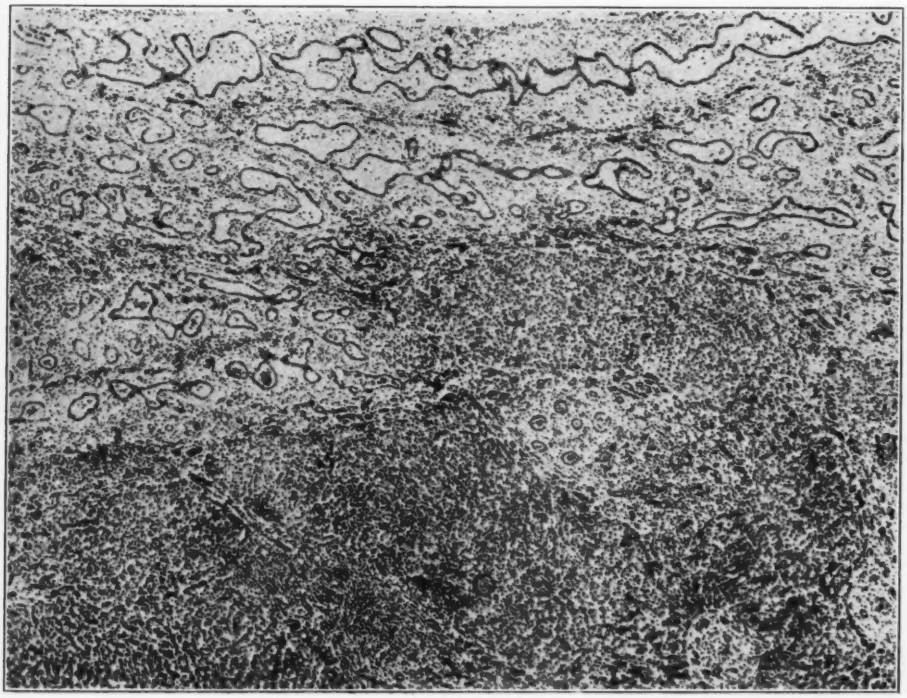

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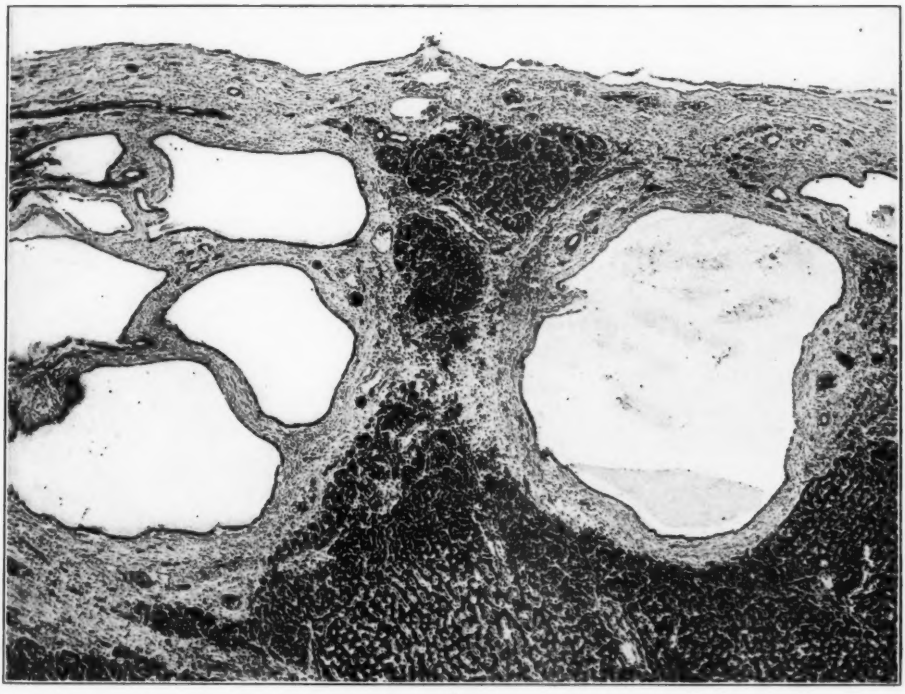
PLATE 103

FIG. 7. Photomicrograph of the liver of an adult showing the same anomaly of the bile ducts. They are increased in number, moderately dilated and lined with a uniform cuboidal type of epithelium. The connective tissue stroma, as in the earlier cases, is also considerably increased. × 100.

FIG. 8. Photomicrograph of the liver from an elderly male. It shows the same bile duct anomaly. Here some of the ducts have become isolated, and as cyst-like spaces are distended with clear fluid. The bile duct epithelium is extremely compressed. The unusual amount of connective tissue surrounding the cysts suggests an old healed inflammatory lesion. × 50.

7



8

MacMahon Congenital Anomalies of Liver

SCIENTIFIC PROCEEDINGS OF THE TWENTY-NINTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

CHICAGO, ILL.

MARCH 28 AND 29, 1929

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

REPORT OF A CASE OF PEL-EBSTEIN'S DISEASE OF TUBERCULOUS ORIGIN. Elise S. L'Esperance, New York City.

(*Abstract not received.*)

SKIN REACTIONS IN SPRUE PATIENTS TO VARIOUS FOOD PREPARATIONS. Charles Weiss and (by invitation) Oscar Costa-Mandry, St. Louis, Mo.

Abstract. An investigation was made to determine whether the untoward gastro intestinal reactions observed in sprue patients (diarrhoea, tympanites, flatulence, etc.,) after eating certain types of food might be of an allergic nature. A careful survey was made of the foods commonly eaten by the people of Porto Rico, and representative types were selected as antigens for intracutaneous tests. These included *proteins* prepared from rice, beans, pork, cod-fish, cocoanut, etc.; *sugars* (saccharose and fructose); *fats and oils* (lard, butter, etc.,); *starches* prepared from rice, potatoes, beans, etc.; and local spices and coffee.

Our results were clearly negative and do not lend support to the hypothesis that the untoward digestive symptoms of sprue patients are due to specific hypersensitiveness to certain food products.

THE ANTIGENIC PROPERTIES OF THE PROTEIN FROM TUBERCULIN. J. H. Lewis and (by invitation) F. Seibert, Chicago, Ill.

Abstract. The proteins used in these experiments were obtained from 6 to 8 weeks cultures of avian, bovine and human tubercle bacilli and timothy bacilli grown on Long's synthetic non-protein liquid medium, and were isolated either by precipitation with ammonium sulphate or by ultrafiltration. The processes used in either case were such that denaturation was prevented. The proteins were readily soluble in water, forming perfectly clear solutions. While they were not crystallized proteins they were the starting point where Seibert began her crystallization of the tuberculin protein. In doses of 0.1 milligram (the smallest amount tested) they were actively anaphylactogenic as proved by intravenous injections of 10 to 15 milligrams into sensitized guinea pigs, by the uterine strip method, and by the bronchospastic method of Lewis and Koessler. Cross-sensitization tests (intravenous and uterine strip) made with avian, bovine and human tuberculin proteins showed that these three proteins give cross-reactions but are distinguishable from each other by desensitization tests. Uterine strips from guinea pigs sensitized with the tuberculin proteins do not react to the protein from timothy bacilli filtrates, and *vice versa*. Guinea pigs injected with the human tuberculin protein produced high titers of precipitins. The sera containing such precipitins could passively sensitize normal guinea pigs in doses as small as one cubic centimeter. Tuberculous guinea pigs died from acute anaphylactic shock when the tuberculin protein was injected intravenously. With the bronchospastic and uterine strip methods it was shown that this true anaphylactic sensitization developed about six weeks after infection. Sera from tuberculous guinea pigs and from some humans with tuberculosis contained high titers of precipitins, but, in contrast to precipitating sera from

normal immunized guinea pigs, they were not able passively to sensitize guinea pigs.

Discussion

(Dr. A. F. Coca, New York City.) The most surprising thing about this very interesting study is the lack of specificity which seems to be shown in some of the earlier experiments, although Dr. Lewis does give one experiment, at any rate, that demonstrates a certain specificity of the protein of the timothy bacillus.

With respect to bovine and human tuberculin, I am not sure whether there is a definite, specific difference in the tuberculin active substance; but between avian and human tuberculin, there is a distinct impression from the experiences of Lowenstein that they are specifically different as tested in the infected individual.

Dr. Wollstein says that recently she has had a considerable series of experiments of that kind carried out on children in New York, and found some of them (something over 100 cases) positive to the human tuberculin, but negative to any concentration of avian tuberculin. There may be some question as to whether these experiments of Dr. Lewis' need to coincide with those carried on in the human being. At any rate they seem to disagree, because I understand that Dr. Lewis believes that he has isolated here the particular substance that is responsible for the tuberculin reaction. There is a discrepancy here that evidently needs further study with reference to the interesting report we had yesterday by Dr. Dienes, who, no doubt, would have something further to say along that line.

(Dr. E. L. Opie, Philadelphia.) I should like to ask Dr. Lewis if these guinea pigs gave skin reactions to the protein that he used.

In an effort to correlate anaphylactic hypersensitiveness and the tuberculin reaction Baldwin and Austrian failed to obtain skin reactions with tuberculin prepared by the usual methods, though they obtained anaphylactic shock by injection of tuberculo-protein into the blood stream or into the central nervous system. They have emphasized the distinction between anaphylactic hypersensitiveness to tuberculo-protein and cutaneous and toxic sensitiveness to tuberculin.

(Dr. Stuart Mudd, Philadelphia.) I think the apparent discrepancy Dr. Coca has pointed out can be explained. Extensive studies of the serology of acid-fast bacteria have shown that avian tubercle bacilli give strong cross-reactions with bovine and human strains; indeed reciprocal adsorption tests may be required to differentiate the avian and mammalian strains with certainty. On the other hand certain saprophytic strains, of which "timothy" is no doubt one, do not cross-react with mammalian tubercle bacilli.

I was surprised though, if I understood correctly, that there should be a distinction between bovine and human tubercle bacillus proteins, because all the work that has been reported and all we have done has failed to show any serological distinction between the human and bovine strains.

(Dr. E. R. Long, Chicago.) The results just brought out are much more striking than those secured by Dr. Baldwin some years ago, which are considered the pioneer work on this subject. It should be pointed out that Dr. Baldwin used a water extract of dried tubercle bacilli, and in the drying process there is no doubt that a considerable amount of change took place. There is much loss of biological activity in drying. These results are probably so much more strik-

ing than Dr. Baldwin's because the materials were subjected to chemical methods not likely to lead to change in the character of the proteins.

I am much interested in what Dr. Coca and Dr. Mudd had to say about the relationship of these microörganisms. I might recall some work which I did some years ago on the tuberculin reaction. I found that animals that were sensitive to human tubercle bacilli and corresponding tuberculin were also sensitive to other organisms of the acid-fast group but not to the same degree. My own feeling is that probably not exactly the same protein, but related proteins are present in the bacilli of this group.

(Dr. E. Pribram, Chicago.) I should like to know whether these animals became immunized because of the high character of the protein and become less sensitive, which affords a protection from infection with the tubercle bacilli.

(Dr. H. Swift, New York City.) I should like to ask whether the skin reactions (just amplifying Dr. Opie's question) were of the immediate type occurring within the first two or three hours or whether they were the late tuberculin type of reaction.

(Dr. L. L. Dienes, Asheville, N. C.) With preparations similar to those used by Lewis and Seibert, we obtained abundant antibody production only in tuberculous guinea pigs, not in normal ones. Complement fixation antibodies were produced in larger amounts than precipitins. In the filtrates of synthetic media we found only the proteins precipitable with acid, antigenic *in vivo*, and this protein fraction is a different antigen from the fraction non-precipitable with acid. We have to pay special attention to the fact that certain protein preparations produce antibodies in abundance toward carbohydrate precipitable substance. The antigenic effect *in vivo* of different preparations varies to a great extent, and possibly this is the reason our observation does not entirely correspond to that of the authors.

(Dr. Lewis, closing.) We studied cross-anaphylactic reactions with two methods, the intravenous injection method and the uterine strip method. With the intravenous injections into sensitized animals it was impossible to distinguish between the three types of tubercle bacillus proteins, since animals sensitized to one died equally rapidly when injected with either one of the other two. We did not try to desensitize living sensitized guinea pigs with non-fatal doses of protein, but did make desensitization experiments with the uterine strip method which lends itself particularly well to this purpose. There is a similarity between the three tubercle bacillus proteins because a uterine strip sensitized to one will react to either of the other two, but after such a reaction there always remains some sensitization to the homologous protein. In other words, the three proteins are closely related but are not identical, since one will not desensitize to the other. Whether the bovine protein is more closely related to the human protein than avian, we cannot say from experiments made so far.

We did not make comparisons of the tuberculin test and the anaphylactic reaction in tuberculous animals because we wanted to avoid any anaphylactic desensitization that might occur from the injection of small amounts of tuberculin.

In animals given several injections of as large quantities of tubercle bacillus proteins as were not fatal, a suggestion of a skin reaction could be elicited which resembled a tuberculin reaction. The impression is given that if the protein was less toxic so that larger and more frequent injections could be given, a degree of skin sensitization could be obtained which would simulate very closely a true tuberculin reaction.

We did not try to study the protection from infection with tuberculosis that injections of these proteins afforded. We were not interested in this problem at that time.

In answer to Dr. Swift's question about the immediate reaction, we considered only as positive anaphylactic reactions those animals that gave an immediate, acute reaction and disregarded those reactions which were delayed and which were fatal the next day, because we could not be sure that the latter type of reactions was due to anaphylaxis or to the primary toxicity of the proteins. We used the same criteria that we use in anaphylactic tests with other proteins. We made no attempt to study the relation of carbohydrates to these experiments. We were aware at all times that our preparations contained carbohydrates. Dr. Seibert states that tubercle bacillus proteins are freed of carbohydrates only after repeated crystallizations. As the immune reactions of these proteins after purification by crystallization will be studied, some facts will be obtained on the rôle of carbohydrates.

ON THE MECHANISM OF OPSONIN AND BACTERIOTROPIN ACTION-ANTIBODIES IN EXPERIMENTAL TUBERCULOSIS IN THE RABBIT. Baldwin Lucké, Morton McCutcheon, Max Strumia, Emily B. H. Mudd and Stuart Mudd, Philadelphia, Pa.

Abstract. Last year we reported that whenever in our experiments acid-fast bacteria were prepared by serum for phagocytosis certain definite changes in the surface properties of the bacteria could be demonstrated; that, furthermore, whenever these bacterial surface changes were found, except under certain artificial conditions, the bacteria were prepared correspondingly for phagocytosis. The bacterial surface changes correlated with the increased phagocytosis are increased cohesion, decreased surface electric potential difference and decreased wettability by oil. In the present experiments we have again found this correspondence between the surface properties and the phagocytosis of bacteria under a set of conditions not hitherto tested, namely experimental tuberculosis in rabbits.

In addition to the above, certain interesting findings concerning the development of antibodies in tuberculosis have come out of those experiments. Three groups of rabbits were infected with virulent tubercle bacilli. Other groups of rabbits had been inoculated six months earlier with 0.001 mg. of virulent human tubercle bacilli; the resulting tuberculosis had largely regressed, leaving only residual foci in the lungs and occasionally in other organs. These second groups of rabbits were reinfected with virulent tubercle bacilli at the same time and with the same dosages as the primarily infected animals. This work was done by Dr. Max B. Lurie. The titers of the sera of both primarily infected and reinfected rabbits were then followed by resuspension, cataphoresis and phagocytosis.

There was found a slight and slowly developing production of antibodies in first infection rabbits which showed a moderate and slowly developing resistance to the infecting tubercle bacilli, and a prompt production of antibodies in quantity in the reinfection animals, which were highly resistant to the reinfecting organisms. This association of heightened resistance with increased capacity for antibody production in reinfection animals suggests, without proving, however, a rôle of antibodies in immunity to tuberculosis.

The development of antibodies causing bacterial surface changes and phagocytosis occurred, within the experimental error, simultaneously.

Discussion

(Dr. E. L. Opie, Philadelphia.) One phenomenon that has been brought out by this study is of especial interest, namely, the capacity of an animal previously immunized to form antibodies with more rapidity on the second immunization than on the first. As Dr. Mudd has pointed out, this has been observed with a number of microörganisms, first by Dr. Cole with the typhoid bacillus. Even after the complete disappearance of antibodies, a second immunization would cause them to reappear more rapidly than the first immunization. This phenomenon suggests that protection is dependent on factors more fundamental than antibody formation.

(Dr. E. R. Long, Chicago.) Am I right, Dr. Mudd, in assuming that the phagocytosis was carried out entirely with polymorphonuclear leucocytes? I wonder if it would not be possible to carry out this test with the large mononuclear cells. While it is probably true that the same mechanism is concerned in the phagocytic activity of each cell, and also true that the polymorphonuclear is of more importance in tuberculosis than is generally conceded, I should think if the experiment could be carried out with the large mononuclear cell that it should be done.

(Dr. Mudd, closing.) I can only agree with Dr. Long. Dr. Opie has brought out the same point repeatedly. That is an early item on our future program. This study has been primarily an effort to get light on the mechanism of opsonin and bacteriotropin action. Immunity in tuberculosis has been a secondary, rather than a primary, concern. Of course, you are quite right about the importance of studying phagocytosis by monocytes.

STUDIES ON THE MECHANISM OF THE REACTION OF ANAPHYLAXIS. Noble P. Sherwood, and (by invitation) Paul E. Davis and Raymond A. Schwegler, Lawrence, Kan.

(*Abstract not received.*)

CROSSED ANAPHYLACTIC RELATIONSHIP BETWEEN HORSE DANDER AND HORSE SERUM. Bret Ratner and (by invitation) H. L. Gruehl, New York City.

Abstract. The crossed anaphylactic relationship between horse dander and horse serum was studied in the guinea pig. Animals were sensitized to horse serum and cross-shocked with horse dander extract. Other animals were sensitized with horse dander extract and cross-shocked with horse serum. The above crossed reactions were further carried out using the Schultz–Dale method. In other groups, animals were sensitized by the nasal inhalation of dry horse dander in specially constructed dust chambers. The cross-shock injection was with either horse serum or diphtheria antitoxin.

The results obtained show a definite crossed relationship to exist. However, animals sensitized with horse dander extract more readily exhibited the crossed relationship when shocked with horse serum than did animals sensitized with horse serum and shocked with horse dander extract. Animals in which respiratory anaphylaxis was produced by normal inhalation of horse dander could be shocked with an injection of horse serum or diphtheria antitoxin.

Discussion

(Dr. N. P. Sherwood, Lawrence, Kan.) I should like to ask Dr. Ratner what amount of serum he used in the bath. I also am wondering about this six

minutes that he mentioned as elapsing between the time he added horse serum to the bath and the response. Such a time element I have never seen where I would call it a specific response. It might be, I presume, but I have never seen a response coming on as a specific response after six minutes.

Spontaneous contractions I have observed often in an irritable horn. If I had just added something before, then I would apparently have a positive reaction. Sometimes they come on after a considerable period.

With regard to the dosage he adds to the bath, I think more errors are included in this smooth muscle work because of not determining the toxic dose, and not realizing that it will respond to it tremendously. For example, if you take ten guinea pigs to determine the non-toxic dose of antigen to be used in the bath, and test out the normal pigs, you will find that perhaps one of the ten will respond to 6 cc. and not 5 cc., whereas another one of the ten pigs will respond to 1 cc. and above. If you took as the control pig, the pig that responded to 6 cc. and added 2 cc., you would think you had a non-toxic dose. It is imperative in this case to rule out the toxic reactions because it is impossible to determine by their appearance whether they are toxic or anaphylactic.

(Dr. W. H. Welker, Chicago.) I should like to ask Dr. Ratner how he explains the cross-reaction. The dander protein seems to be entirely different from the blood protein. In one case of extreme sensitization with dog dander, we had occasion to study this point. The water-soluble protein obtained by washing dogs gave a marked skin reaction. When immunization was carried out, the simple moisture that was on the needle caused a severe reaction. We were unable to get the slightest response on the part of any of the blood proteins of the dog. Does Dr. Ratner believe that in the case of this horse dander cross-reaction enough of the serum protein has permeated through the skin so that cases which are extremely sensitive to the serum proteins might give this response, and those others that are not particularly sensitive are not sufficiently sensitive to respond to such an extremely small amount of blood protein?

(Dr. Ratner, closing.) In answer to Dr. Sherwood, we used the 250 cc. in the bath. The question of delayed reaction, I think, is quite in order in so far as we get delayed reactions in anaphylaxis depending to a great extent on the solubility of the substance used. It is possible that the common substance in our solution had to find its way out of the complex protein combination, which may account for the somewhat delayed reaction.

As Dr. Lewis showed a number of years ago, when you use small amounts of protein combined with large amounts there is a masking of the lesser portions, so with our solutions there are present many more proteins than would be evidenced by the common factor.

The question of toxicity, I suppose, is a very classic question. We have made innumerable controls, and we do not use anywhere near the toxic dose. One cc. is the amount we use, and that amount has never produced primary toxicity in our hands.

In answer to Dr. Welker, the question of serum getting into the dander has been raised. Dr. Park particularly called my attention to that. It has been possible that in currying the horse, horse serum does get in. We used varying types of horse dander over a period of four years, getting them at various times of the year, and we believe if this is a factor, the same factor holds true with the human problem.

We believe, however, that there is a common substance, probably a globulin, for anaphylactic death was obtained with antitoxin. We used the preparation

obtained from the New York Department of Health which is supposed to contain only pseudoglobulins of horse serum.

ANTIGENIC RELATIONSHIPS OF A STRAIN OF EBERTHELLA PARADYSENTERIAE AND A STRAIN OF SALMONELLA MORGANI ISOLATED DURING AN EPIDEMIC OF SUMMER DIARRHOEA. G. M. Mackenzie and (by invitation) Louise N. Batt, Cooperstown, N. Y.

Abstract. During a small epidemic of dysentery characterized by blood and mucus in the stools, five cultures of an organism belonging to the paradysenteriae group (closely related to the Flexner type) and four cultures of organisms of the *B. Morgani* group were isolated. By fermentation reactions, agglutination and reciprocal absorptions the five paradysenteriae cultures were found to be antigenically identical. The four cultures of *B. Morgani* no. 1 were found by the same methods to represent three antigenically different varieties. Reciprocal absorption tests revealed an antigenic relationship between one strain of *B. Morgani* no. 1 and the paradysenteriae strain, and a common antigenic component. Cultivation of a single cell culture of the paradysenteriae strain in 5 per cent serum (homologous) broth with transfers every five days resulted in the appearance of two stable variants. One variant is inagglutinable in its homologous serum and in all other serums tested. The other variant agglutinates to titer in serum prepared from the mother culture and in *B. Morgani* serum, but does not agglutinate in any of the other heterologous serums available. Reciprocal absorption tests with the second variant, its homologous serum, *B. Morgani* and its homologous serum, show a common antigenic component. Other modifications of the mother strain apparently permanent in the variant are altered appearance of the colonies, acquisition of the capacity to form indol and to ferment maltose and saccharose. The possible bearing of these results on the pathogenicity of *B. Morgani* and the relationship between *B. paradysenteriae* and *B. Morgani* are discussed.

CINEMA DEMONSTRATION OF THE EFFECT OF FILTRATES OF CULTURES OF THE COLON-TYPHOID GROUP OF ORGANISMS ON THE MOVEMENT OF RABBIT INTESTINES. E. E. Ecker and (by invitation) M. A. Biskind, Cleveland, O.

REFINED ANTI-POLIOMYELITIS. Elliot R. Weyer (by invitation), William H. Park and Edwin J. Banzhaf, New York City.

Abstract. Landsteiner and Levaditi discovered in 1910 that monkeys which had recovered from poliomyelitis had neutralizing bodies for the virus in their blood. Netter showed that the blood of convalescent human cases also contained them. Netter in 1910 claimed that the injection of convalescent serum into cases of poliomyelitis even as late as twenty-four hours after the development of paralysis was beneficial.

The thought that horses might be immunized so as to furnish a plentiful and reliable supply of potent serum occurred to several. In 1910 Flexner tried to immunize a horse by injecting it repeatedly with an emulsion made from the potent cord and brain of the monkey. He tried but one horse and with disappointing results. He states "the blood serum of a horse after many months of treatment had no restraining effect on the virus either *in vitro* or within the body." In 1916 Neustaedter and Banzhaf made a new attempt to produce a potent horse serum. They first tried to prepare a vaccine by digesting the in-

fected cord and brain substance with trypsin. This was unsuccessful. They then changed the vaccine. A 5 per cent emulsion of the cord and brain substance of monkeys was prepared and filtered through paper pulp. This material was injected every two days. The subcutaneous injections beginning with 50 cc. were increased so that the eleventh injection was 300 cc. The horse then for the first time seemed decidedly ill. A bleeding was taken. The horse died the next day. This serum had decided antivirus potency. A control monkey given 0.5 cc. per cent emulsion of virulent brain substance died on the seventh day of poliomyelitis. Another monkey receiving the same dose was given twenty-four hours later 3 cc. of the serum intraspinally and 17 cc. intramuscularly. These injections were repeated on the two following days. The monkey remained well. A monkey in which the injections were begun forty-eight hours after the inoculation was not protected. Another monkey received an inoculation into the brain of a fatal dose of virus mixed with 0.6 cc. of the horse serum. This monkey survived. At the time and later, this serum was used in a number of human cases with doubtful success.

In 1917 Pettit, in the Pasteur Institute, reported that he had succeeded in developing a potent serum in sheep and, in 1919, in horses. He states that he was unable to estimate the potency of the serum because of lack of monkeys. The serum was used rather extensively in France with apparent success. Even late cases were thought by Stefanopoulo to be benefitted.

Stimulated by the good reports of the use of convalescent serum in early human cases recently reported by Aycock and having a large amount of virulent monkeys' brains on hand, we began again the extensive treatment of a horse, giving the injections of the emulsion every few days intravenously. After four months of injections we allowed the horse to rest for six weeks and then took three full bleedings. To our surprise we found the serum of the first two bleedings to be somewhat more potent than the average human serum from convalescent human cases.

One of us, Banzhaf, took two liters of the serum and refined it in the following manner: To the serum he added an equal quantity of water. The diluted serum was saturated with sodium chloride. The resulting precipitate was filtered off. To the clear filtrate a saturated solution of ammonium sulphate was added up to 33 per cent to precipitate the pseudoglobulins. This precipitate was pressed to remove the excess of salts and then dialyzed free from the remaining salts. To the dialyzate was added 1 per cent of sodium chloride and trikresol as a preservative. The globulin solution was then finally cleared by passing through paper pulp and Berkefelds. The 2000 cc. of the serum yielded 350 cc. of globulin solution. Fifty cc. of this concentrated pseudoglobulin solution was added to a quantity of distilled water as is done in refining the antipneumococcic serum with the hope of making a further purification. As will be seen in the figures given on the slides, the first step in refining was successful but not the second step.

(Dr. A. B. Wadsworth, Albany.) I presume you have not had a chance to determine the length of time the serum retains its potency or whether there is any loss, or a lack of stability due to the concentration. Sometimes these concentrated products are not quite as stable as the original sera.

(Dr. Weyer, closing.) So far we have carried on the test on serum stored for only one month. In that time it apparently had lost nothing, so it seemed that we had a stable, refined serum as far as any serum could go.

THE LYMPHATIC TUMOR REGISTRY PROGRESS REPORT. G. R. Callender, Washington, D. C.

Abstract. The following case of general lymph node enlargement terminating in a sarcomatous type of Hodgkin's disease represents one of the types which is of especial value to the study for which the registry of lymphatic tumors was instituted by the American Association of Pathologists and Bacteriologists and is presented with a view to stimulating interest in that registry.

Patient born in August, 1889. Mother and two brothers have had tuberculosis but are living. Acne vulgaris of face at age of 20 to 26 which was much improved by X-ray treatment in his 26th year. In this year he noticed enlarged nodes in his axilla and groin. These were not tender or painful. At the age of 31 he had an attack of acute gastro-enteritis. At the age of 32, there was noticed a general glandular enlargement, the largest node being in the left groin. This was stated to be of the size of an English walnut. This node was removed for examination. Spleen was not palpable at this time. Many pathologists have seen this node, and the majority have considered that it was not a tumor. There appeared to be an increase in number of the lymph nodules (follicles) which varied markedly in size, and there were moderately numerous mitotic figures. There were no cells of the Reed type. The cells of the follicles were closely packed, fairly uniform in size and stained deeply. The blood was reported normal at this time. X-ray treatment reduced the size of the nodes.

At the age of 35 he had a severe attack of gastro-enteritis, cause undetermined. At this time there was greater general glandular enlargement than at the age of 32, and the spleen extended to the iliac crest. X-ray treatment reduced the nodes which did not enlarge again and decreased the size of the spleen. There was a severe prostration following this X-ray therapy from which he did not recover for several months. The nodes removed at this time are said to have been like those of the biopsy at the age of 32. One of them was reported to show tuberculosis, but this has not been verified. At the age of 37 an attack of intestinal indigestion lasted three days. At the age of 38 he was in quarters five days with "influenza."

His final illness began in June, 1928, at age of 39 years. The onset was sudden with temperature 103° to 104° F. There were dull aching pains in the left lumbar region. He was dull, and his sleep was stuporous. He partially recovered from this acute phase and went on leave but was forced to return to the hospital because of irregular weakness in his extremities and back pain. He gradually failed, showing symptoms of brain and cord involvement and died in October, 1928.

At necropsy the superficial nodes were not palpable. The spleen and deep abdominal nodes and thoracic nodes were enlarged. The histological picture is that of a very cellular, invasive Hodgkin's disease, with vascular invasion.

During the final illness the lymphocytes in the peripheral blood varied in number, the lowest being 22 per cent with 5650 leucocytes; the highest 61 per cent with 6300 leucocytes.

Discussion

(Dr. J. Ewing, New York City.) The work of the Lymphatic Tumor Registry seems to be very much needed in this country, and indeed in this Association. Quite variable views have been expressed by members of this Association regarding the diagnosis and prognosis of many cases sent to the Registry. The

existence of the group of lympho-epithelioma metastatic in the lymph nodes is not generally recognized, and these cases are often mistaken for lymphosarcoma, from which they may be very difficult to distinguish. Variations in the structure of Hodgkin's nodes are numerous and difficult to trace and evaluate. We all have to admit that our knowledge of many lesions in lymph nodes is quite inadequate to enable us to make a safe diagnosis and prognosis. I believe that a Registry of Lymphatic Tumors is more important practically than the Registry of Bone Sarcoma. I trust that the members of the Association will take the little time and trouble necessary to register all their significant cases, so that within a reasonable period the Registry will contain material enough to settle many difficult questions of diagnosis and prognosis.

(Dr. H. G. Wells, Chicago.) In your request for material of which we have an early specimen concerning which there has been doubt, do I understand from that, that you do not care for records of cases first seen at autopsy? Or, would these also help your collection?

(Dr. Callender.) I did not say, perhaps, just what I meant. I have been asking the members of the Association to send in all their cases. That has not been successful. Now I am begging them to send in those which are especially difficult and peculiar. It won't make so much work, and perhaps we will get farther that way than by trying to get them all in. I have asked you to send all of them in, including the many hundreds that some of you have, as we want them all. So I am trying to get particularly the group of cases that offer difficulties in diagnosis at biopsy, which we can follow through to the end.

(Dr. E. B. Krumbhaar, Philadelphia.) I might add that Major Callender will even treat very kindly, material that really does not belong in the lymphatic registry at all. I know this because I sent him one myself, and there was no great harm done. So, if you are in doubt, I should say to send it along. If you don't happen to remember the exact address, probably if you just send it to Major Callender, Washington, D. C., that would be sufficient. I hope you will all take some of these blanks with you, and you will see that you don't have to make a very complete report in order to satisfy his requirements, although the more complete it is, the better.

(Dr. A. S. Warthin, Ann Arbor.) I will say just a word. I am one of those who has decided views on this subject. If Dr. Callender wants a diagnosis on his case of sarcomatous Hodgkin's, I would offer it as a typical example of a tumor of this type. We have had many of them, and I feel there is no very great difficulty in the diagnosis. Being in this game for about thirty-five years, I have classed these various neoplasms on rather hard and fixed lines, trying to show their genetic relationship; and I have become convinced that Hodgkin's, the various forms of sarcomatous Hodgkin's, of reticular sarcomas of the lymph nodes, the various aleukemic and leukemic lymphoid tumors are genetically related one to another, and form a distinct class of neoplasm.

(Dr. Callender, closing.) I think I have covered Dr. Warthin's last statement. The difficulty was in the diagnosis of the case from the biopsy made in 1921. About seventeen pathologists have seen this node, and most of them, including myself, considered it a simple hyperplasia.

At the end there was no difficulty in diagnosing the case from the necropsy material. Only one man diagnosed Hodgkin's disease on the biopsy material, and I cannot see even now why he did it.

DIAGNOSTIC RELIABILITY OF FROZEN SECTIONS. William Carpenter MacCarty, Rochester, Minn.

Abstract. Frozen sections have been used routinely in the Mayo Clinic for twenty-three years, during which time 208,255 surgical specimens including 28,833 cancers have been studied and diagnosed during operations. Proof of the correctness of diagnosis is controlled readily by subsequent removal of the whole specimen and by the subsequent clinical history. All cases have been followed, and the efficiency of the method definitely established. In fact the freshness of the material and the lack of artifacts such as naturally occur in postmortem embedded material permit more efficient use of an oil immersion lens. By this means the detailed character of the cells becomes more apparent.

In conclusion it may be said: Surgeons and other clinicians know the value of frozen sections, but many professors of pathology who have been depended upon to teach surgical pathology and furnish candidates for positions in surgical pathology have failed to investigate thoroughly frozen section methods. The surgeon, being a very practical man, must of necessity build up specially trained surgical pathologists, who will be divorced unfortunately from the field of general pathology.

Pathologists, if interested at all in tissues, are missing a great opportunity to see cells as they are; they are failing to improve their understanding of their own subject. By their lack of clinical interest and the failure to utilize fresh tissues they are rapidly becoming known to be less efficient as microscopic diagnosticians.

My plea to you is that you open your eyes to a method which will startle you after a little practice. "I have never seen such beautiful cells" is the usual comment when properly made frozen sections are demonstrated to pathologists who are accustomed to see only embedded material.

AN IMPROVED MODIFICATION OF THE WARTHIN–STARRY TECHNIQUE FOR THE STAINING OF SPIROCHETA PALLIDA IN TISSUE SECTIONS. A. S. Warthin, Ann Arbor, Mich.

Abstract. Eight years experience with the Warthin–Starry staining method for the demonstration of spirocheta pallida in single sections has shown that it is the most rapid and most efficient method for the staining of spirochetes yet published in the literature. It can be used for all tissues except brain and cord. By its use spirochetes can be demonstrated in about 60 per cent of tissues containing histological lesions of syphilis. Nevertheless, it behaves capriciously with many fixations, requiring individual modifications in order to bring out the spirochetes present. Because of this fact, this laboratory has constantly aimed at the improvement of this method. With the present modification a much higher percentage of successful staining of poorly fixed tissues, even alcoholic fixation, has been obtained. It is, therefore, highly recommended for use in the case of all tissues except the central nervous system, for which we advise the use of Dieterle's method.

A COMPARISON OF METHODS OF STAINING SPIROCHETA PALLIDA IN TISSUE. R. E. Olsen (by invitation), Ann Arbor, Mich.

Abstract. Six different tissues, each containing the spirochetes of syphilis in large numbers, were stained by six methods of demonstrating the organism in single sections. Four of the six tissues were impregnated by three block methods. All the stains used were silver nitrate reductions. The methods used were:

Levaditi, Haythorn, Jahnel, Warthin–Starry (unmodified), Warthin-Starry (nitric acid-starch modification), Wright, Dieterle, Gyenes and Sternberg, and Armuzzi and Strempel. Three of the tissues used were from congenital syphilitic material, and three from acquired syphilitic cases. The results obtained show that while no method is absolutely dependable certain stains are better suited to selected tissues than others. With few exceptions, if the spirochetes can be demonstrated by the use of silver nitrate it will be best done by the improved Warthin–Starry method or that of Dieterle.

Discussion

(Dr. E. B. Krumbhaar, Philadelphia.) Dr. Warthin's and Dr. Olsen's papers are now open for discussion.

I should like to ask Dr. Warthin if opportunity has been offered by any of the methods used to get an idea of how often spirochetes may be found in adult syphilitic conditions, particularly in such cases as one would customarily consider at autopsy to be syphilitic myocarditis or aortic valvulitis.

I should also like to suggest to those who perhaps are not quite so successful as Dr. Warthin with these methods to remember before they criticize too freely the sign the Germans put up in Albert during the World War, "Nicht ärgern, nur verwundern."

(Dr. Warthin.) Do I understand you to ask me how often we have been able to demonstrate spirochetes in the myocardium?

(Dr. Krumbhaar.) What you have considered as being spirochetes in valvulitis.

(Dr. Warthin.) With the old method, about 50 per cent of the cases. With the new method, the percentage will be higher. We have not restained all the material which has been negative for spirochetes, but which shows histological lesions. We have recently had a heart sent to us from Atlanta, Georgia, a heart in advanced decomposition, alcohol fixation; and yet, with my new method, we were enabled to find spirochetes in the typical lesions. I consider that a very great victory for the method, because with my first method, I have been emphasizing that the tissue must be just as fresh as possible; that you stand no chance at all with postmortem changes, and with alcohol fixation. With this new method, by freshening up the tissue with nitric acid, and using the new silver-starch combination, we had beautiful staining of spirochetes in this heart. The new method has not been developed long enough for us to control a large number of hearts that have histological lesions in which we were not able to find the spirochetes by the old method.

(Dr. Krumbhaar.) I understand that, for instance, in the ordinary aortic valvulitis at autopsy in syphilitic organs you expect to find spirochetes in 50 per cent of the cases.

(Dr. Warthin.) In about 75 to 80 per cent with this new method. It is not a 100 per cent perfect method.

(Dr. S. R. Haythorn, Pittsburgh.) In view of the comparatively poor showing of my method as indicated in the photomicrograph just shown, I feel called upon to say a few words in its defence. I think that these methods probably stain much better in the hands of those who work with them constantly than in those who use them only occasionally. In general, my method has yielded very satisfactory results, and I have shown some very clear photomicrographs of spirochetes stained black by it. I have tried Dr. Warthin's former method, and I was not at all successful with it in many instances. We still use my method

in our laboratory, and are well satisfied with it. But I shall be very glad to try Dr. Warthin's new technique in comparison. I am very sorry that it does not work on brain tissues, because that has been one of my big problems.

(Dr. Olsen.) I am glad the personal element was emphasized. I think that is very true. However, I was careful to point out that since we had used the Warthin–Starry method so long we should be considered more expert in that than other methods. Perhaps had we used Dr. Haythorn's method as often as we had used the other, we would get better results.

(Dr. Warthin, closing.) The personal element cannot be over-emphasized because with the Warthin–Starry method we have been able to get results in tissues where other people to whom we have taught the method and shown the method have failed absolutely to get anything. It is just an example of an individual learning the tricks of this stain. These stains are tricky as they can be, because you have to vary the amount of nitric acid and the length of time nitric acid is used, just as before we had to experiment with uranium and hydrogen peroxide, by actually coaxing the tissue. It is largely a question of the hydrogen ion concentration of the tissue.

You are able to get results, but sometimes you can't explain those results. I have had several cases of umbilical cords showing very marked histological lesions, and yet with my old method we could not get a single spirochete. Just the little trick of soaking it in nitric acid changed the tissue reaction and made the spirochetes relatively more basic in the tissue so they precipitated the silver. With such temperamental characteristics on the part of the stain, the great disparity of results produced by different workers is not to be wondered at. I think the explanation of the greater number of positive results with us is simply that we learned the temperamental characters of the stain and experimented with them until we got results.

DEMONSTRATION OF RICKETTSIA IN SCROTAL SAC EXUDATE IN EUROPEAN TYPHUS GUINEA PIGS. Henry Pinkerton, Boston, Mass.

Abstract. Because of Mooser's observation of a minute intracellular diplobacillus in the scrotal sac exudate of guinea pigs reacting to Mexican typhus, attempts were made to obtain a similar picture in European typhus guinea pigs. These attempts were at first unsuccessful because observations on the scrotal sac were made at too late a stage in the course of the disease.

Mooser's observations in Mexican typhus were entirely confirmed. Mexican typhus differs from European typhus in that the scrotal reaction is much more severe, the incubation period more irregular (frequently as short as two to three days), and in the rarity of the characteristic brain lesions.

On careful observation, a slight transient scrotal reaction is occasionally seen in European typhus pigs, after intraperitoneal inoculation. If these pigs are killed on or just before the first day of fever, a gelatinous exudate is almost invariably found in the scrotal sac. Rickettsia-like organisms, similar to and probably nearly or quite identical with those described by Mooser in Mexican typhus, may be found in this exudate in European typhus pigs. There appears to be a preliminary multiplication of the virus in the scrotal sac. In the female pig, this multiplication takes place in the general peritoneal cavity, and the organism may be found in a thin layer of exudate appearing on the surface of the spleen. The exudate in the scrotal sac and the scrotal reaction occur only after intraperitoneal inoculation. This separates the disease widely from Rocky

Mountain Spotted Fever, in which the scrotal reaction is independent of the route of introduction of the virus.

Numerous brain lesions may be found in Mexican typhus if the scrotal reaction is prevented by inoculating a small amount of virus subcutaneously. In this way Mexican typhus may be made to resemble European typhus in every respect, but on subsequent passage the disease reverts to type and the scrotal reaction occurs.

The reasons for regarding the organisms as Rickettsia or as the etiological factor in the disease are: (1) their morphological resemblance to the organisms seen in the lining cells of the gut of the European typhus louse; (2) their remarkably weak affinity for dyes; (3) the failure to cultivate the organism; (4) the fact that a minute amount (0.01 cc.) of the exudate in which they are seen suffices to infect a normal pig; (5) the failure of both the microörganisms and the exudate to appear when a typhus immune pig is substituted for a normal pig.

Discussion

(Dr. R. D. Lillie, Washington.) Dr. Maxcy in the Hygienic Laboratory has recently published our observations on a similar Rickettsia, if I may use the term, in the scrotal sac of the guinea pig infected with typhus from the Southern States of the United States, which condition varies in some particulars, both from European and Mexican strains.

I have a photomicrograph showing organisms which, I think, you will consider quite similar to those that Dr. Pinkerton has just shown to us in European typhus and similar to those which Dr. Mooser also showed to us last year at Washington.

(Dr. E. Pribram, Chicago.) I should like to know how you removed the cedar oil.

(Dr. Pinkerton, closing.) Simply wash the cedar oil off with xylol and restain as before.

Symmetrical Osteoporosis of the Cranium. Herbert U. Williams, Buffalo, N. Y.

Abstract. This condition is confined to the outer surface of the skull, chiefly of the parietal and frontal bones. The sutures are usually avoided. The outer table may be altogether wanting or perforated with holes up to 2 mm. in diameter. The cancellous bone is level with the surface or may protrude a little. The surface appears like pumice stone or fine sponge. It was described first by Adachi under the name of cribra parietalia. It is not related to cribra cranii, a somewhat similar condition of the inner surface of the skull. It may be related to cribra orbitalia which affects the orbital plate. Symmetrical osteoporosis may also occur on the occipital, less often on the temporals and sphenoids, very rarely on the face. There are very few reports as to the rest of the skeleton; such information as we have indicates that the long bones were rarely affected. The histological changes in sections made from the parietal bones of two cases consisted of diploë with large spaces for the marrow that opened widely on the surface; in one of them osteophytic growths of similar character protruded above the surface. Sections of ribs, from two other cases, where osteoporosis of the skull was well marked, showed the spaces of the cancellous bone to be unusually large but the outer surface was not affected and the ends of the ribs did not appear like those of rickets. All of these were in young Italian children; the soft parts were missing. Apparently an early acute stage might be followed by heal-

ing, leaving the skull permanently altered. A few cases have been seen in Indonesians. It occurred among the ancient Egyptians, but I am unable to determine how frequently. Todd informs me that he has seen it once in six hundred modern Negro skulls. If it occurs in the modern white race it must be very rare, which explains why the condition is little known among general pathologists. It was frequent among the ancient Indians of Peru, Yucatan, New Mexico and Arizona. That region is also the area in which the cultivation of Indian corn or maize was most highly developed by the Indians before the arrival of white men, and Indian corn is said to be lacking in vitamin C and probably in vitamin D. It has occurred to me that the occiput of the Indian baby commonly rested on a head board, and that may have produced a congestion of the top of the skull by impeding the outflow from the veins of the occipital region. Symmetrical osteoporosis may be allied to rickets. However the changes that we usually consider characteristic of rickets have not thus far been reported in cases of symmetrical osteoporosis. Such information as we have indicates that rickets was a rare disease among ancient and primitive peoples. Autopsies on modern Indians might well throw light on the nature and causation of symmetrical osteoporosis. The two X-ray plates that I have seen made from dried skulls showing this condition give striking pictures that might aid in the diagnosis of living cases. The X-ray shows radiating lines like bristles at the level of the outer table that are evidently produced by the new growths of bone in that situation. (Later note: I have recently learned that similar X-ray pictures have been seen a few times in certain anemias of children.)

Discussion

(Dr. A. S. Warthin, Ann Arbor.) I should like to ask Dr. Williams what criteria he uses for diagnosis that these bone changes are pathological and how he would differentiate them from acid soil erosions or from ant lesions.

(Dr. Percival Bailey, Chicago.) In the Peabody Museum at Harvard University, there is a large collection of such skulls from the abandoned Maya towns in Yucatan, and Dr. Sosman of the Brigham Hospital has made excellent radiographs of several of them.

(Dr. I. P. Lyon, Buffalo.) I should like to ask Dr. Williams if he noticed this condition among the flathead Indians, where pressure had been conspicuously applied to the skulls?

(Dr. D. P. Seecof, Denver.) I recall an autopsy on a white man from New Mexico who died of generalized lymphosarcomatosis. The X-ray pictures of the skull and the gross appearance of the calvarium, if the soft tissues had been removed, would be identical with those shown by Dr. Williams. I shall be glad to send the skull to Dr. Williams. Of course it is a single case, and may have little to do with the cases he reported, but the striking similarity of the gross and microscopic pictures made me bring up the subject of sarcoma.

INTRACRANIAL CHORDOBLASTOMA. Percival Bailey, Chicago, Ill.

Abstract. Discussion of the origin, structure, and symptomatology of intracranial chordoblastoma, with the report of two cases, one verified at necropsy, and the other at operation.

TRAUMATIC PORENCEPHALY. R. H. Jaffé, Chicago, Ill.

Abstract. The pathogenesis of porencephaly is still a matter of discussion and though numerous cases have been reported a few of the publications only deal

with the microscopic anatomy of this condition. The traumatic theory seems to be the most satisfactory one, and additional proof is yielded by the study of the brain of a woman aged 22 years, who since her earliest childhood had been suffering from epileptic fits. A large cystic defect was found in the left occipital lobe communicating with the lateral ventricle and covered by a thin membrane. This membrane was not simply formed by the leptomeningi as has been suggested in several similar cases but was composed of glia tissue with nerve fibers. Myelin sheaths could not be detected. A striking feature was the presence of many dark brown intracellular pigment granules, most of which were found to be composed of hemosiderin. By inquiry, it was learned from the relatives that the patient immediately after birth was dropped to the floor. The accident was followed by unconsciousness that lasted for several hours. The epileptic attacks started right afterwards.

Discussion

(Dr. E. R. LeCount, Chicago.) I should like to recall to Dr. Jaffé's attention, that warty papillary overgrowths of neuroglia with an arrangement of the cells suggesting rosettes were in the tissue lining the defect in one of the porencephalic brains studied by Dr. Semerak (*Arch. Neurol. & Psychiat.*, 1925, xiv, 365–383).

THE PARS INTERMEDIA OF HYPOPHYSIS IN MAN AND IN ANTHROPOID APES. Alfred P. Plaut, New York City.

Abstract. The hypophysis cerebri usually is believed to consist of three portions, namely the anterior glandular part, the pars intermedia, and the posterior nervous part. The discussion on the functions of the three different lobes is far from being closed. The activity of extracts prepared from the different lobes has been foremost among the arguments. The intermediate lobe has been held to be a "metabolism gland," its disorders leading to Froelich's disease. In 1922 the author pointed to the smallness and morphological insignificance of the pars intermedia in the adult human being, asserting that an organ which looks so rudimentary and is so variable in normal individuals can hardly have an important function. In the same year it was found that in a chimpanzee the pars intermedia was as rudimentary as in man, while the few descriptions of the hypophysis in monkeys show a pars intermedia similar to that of other mammals. In a complete series of the hypophysis of an orangoutang the pars intermedia is found even more rudimentary.

Pending investigations on other apes, monkeys and lemurs it can be stated that the hypophysis of man and apes differs essentially from that of other mammals, notably of our laboratory animals. An active principle, found in the pars intermedia of some mammal, probably is not present in the human being. Since the hypophysis plays an important rôle in growth, development and maintenance of body proportions, and in sex life this peculiarity of the hypophysis, common to man and apes, may claim anthropological and zoölogical interest in addition to its importance in endocrinology.

SARCOMA OF THE FLEXOR MUSCLES OF THE LEFT LEG. Edwin F. Hirsch, Chicago, Ill.

Abstract. A malignant tumor arising in the flexor muscles of the left leg produced extensive metastases in the lungs and lymph glands. A groin lymph gland removed several months before death contained long spindle-shaped cells with cross-striations. In the extensive secondary growths obtained for study by the postmortem examination there were cells of primitive or abortive muscle

structure with acidophilic cytoplasm, with an ectoplasm resembling a sarcolemma, with one or more vesicular nuclei, and with concentric fibrils having alternating light and dark bands or with protoplasmic prolongations having longitudinal fibrils and cross-striations like the light and dark bands of skeletal muscle. The cells in many of the metastases contained large quantities of glycogen.

The complete report will be published in the *Archives of Pathology*.

RECONSTRUCTION STUDIES ON BLOOD VESSELS OF THE SPLEEN. Ward J. MacNeal, New York City.

Abstract. Human spleen, perfused and fixed in the distended state has been used to prepare serial sections at a thickness of 5 microns. Drawings at a magnification of twenty diameters have been made on paper, and the drawing of every tenth section transferred to a wax plate 1 mm. thick for preparation of a model showing the general relations of the larger blood vessels and the follicles.

A second series of drawings at a magnification of 200 diameters has been made, and the drawings of every section transferred to 1 mm. wax plates for preparation of a model of the arterial vessels of a single lobule.

The models are not finished. However the work has progressed far enough to show that the splenic lobule can be clearly defined by the distribution of the arterial vessels, which are, in general, terminal for each lobule. Arterial communication at the periphery of the lobule is extremely rare, having been observed only twice, so far, in our preparations.

PATHOLOGY OF SICKLE CELL ANEMIA. Bernard Steinberg, Toledo, O.

(*Abstract not received.*)

ABNORMAL HEART: AORTA FROM RIGHT VENTRICLE, ABSENCE OF PULMONARY ARTERY, DEFECTIVE SEPTUM: DEATH ON THE ELEVENTH DAY. Milo K. Miller (by invitation), and M. W. Lyon, Jr., South Bend, Ind.

Abstract. Report of congenital malformation of the heart. Complete absence of pulmonary artery, the aorta arising from the right ventricle, and the left ventricle communicating with the right by a circular opening beneath the right posterior cusp of the aorta. Lungs supplied by two relatively large bronchial arteries from the descending aorta. Heart enormously hypertrophied, especially the right side. Pressure on esophagus by heart interfering with nutrition. Constant slight cyanosis with severe exacerbations increasing in progress on twelfth day, resulting in death. Hypertrophy of heart, especially of right side, shown by roentgen-ray examination.

Discussion

(Dr. Lyon.) There was no vessel at all leaving the left ventricle. The ventricle itself was really hypertrophied. It had to force the blood out against the contraction of the right ventricle at the same time, it was hypertrophied; but not so much as the right ventricle. The left auricle had four openings into it for the pulmonary veins.

(Dr. B. Halpert, Chicago.) I should like to ask whether the pulmonary artery or the veins connecting showed any anomaly at this time.

(Dr. Lyon.) The pulmonary artery was lacking, but the pulmonary veins seemed to be perfect as far as I could see. The specimen was taken out rather hurriedly, and some of the finer details were missed.

(Dr. E. B. Krumbhaar, Philadelphia.) I am sorry that Dr. Maude Abbott is on her way to Europe so that she cannot participate in this discussion. May I ask the condition of the left ventricle? Of course, there was much atrophy. Was there no sign of even a rudimentary vessel leaving?

PERSISTENT RIGHT AORTIC ARCH IN ADULTS — TWO TYPES. Aaron Arkin, Chicago, Ill.

Abstract. A new form of aortic anomaly is presented. It consists of a total persistence of the right aortic arch, together with a persistence of the left aortic arch in the form of the left subclavian artery, a short obliterated vessel and a diverticulum of the aorta. The patient presented a characteristic X-ray picture due to the position of the arch of the right-sided aorta behind the trachea and esophagus. The diagnosis was confirmed at autopsy. The heart was normal. A new X-ray picture is thus established and confirmed postmortem. Three other cases have been seen by the writer. The anomaly is of great embryologic and clinical interest.

A second type of persistence of the right aortic arch is also reported. Here the aorta rides the right bronchus, the arch remains on the right side, with the left descending aortic root behind the esophagus.

PERIARTERITIS NODOSA — HISTOLOGIC CHANGES IN EARLY AND END-STAGE. Aaron Arkin, Chicago, Ill.

Abstract. Periarteritis nodosa is a specific infectious disease probably due to a filterable virus, with an elective affinity for the arteries of the body. The organs most commonly involved are the kidneys, liver, heart, muscles, peripheral nerves, and gastro-intestinal tract.

In one acute case the writer has found as the earliest changes, periarteriolar hemorrhages due to hemorrhage per diapedesin. In other small arterioles edema with a fibrinous exudation occurred, even before leucocytic infiltration.

In another case, of a patient who had only one acute febrile attack four years before death occurred, only the histologically healed end-stage was found. Cardiac and renal insufficiency were the cause of death. At autopsy hepar lobatum, high grade coronary stenosis, contracted kidneys and myomalacia scars were found. The arterial changes were quite remarkable, with intima proliferation, extensive arterial wall destruction with aneurysms and thrombosis, periarterial granulation tissue mantles, infarction in various organs. No acute inflammatory arterial changes were found in any of the organs.

Discussion

(Dr. A. B. Wadsworth, Albany.) I am interested in the lesions that have been described and wonder whether or not there is any information regarding the bacterial etiology of the arterial changes that have developed from this condition, which appears to be an inflammatory process. These lesions resemble the lesions we find in immunized horses, especially those dying after, or killed during, immunization with the pneumococcus. Without detailed analysis, these pictures are quite similar, not only the hemorrhages of the early lesion of the practically normal vessel but also the obliterating processes in the later stages.

It might be of interest to compare these two inflammatory processes. I described some of the lesions in the *Journal of Medical Research* (*J. Med. Res.*, 1918–19, xxxix, 279), but not with particular attention to the arterial changes, although they are shown in some of the illustrations.

(Dr. E. R. LeCount, Chicago.) Perhaps Dr. Arkin is aware that an example of periarteritis nodosa that was quite well healed was recently found in the County Hospital here.

With regard to the little hemorrhages or partial zone of hemorrhage about one of the vessels in the brain, I should like to suggest simply for the thinking of all of us here that possibly such hemorrhages are caused by handling the brain after death. They are frequently found without any tissue reaction at all about them.

(Dr. E. B. Krumbhaar, Philadelphia.) Eighteen months ago I happened to be in Professor Boycott's laboratory in London while he was doing an autopsy on a somewhat similar case. I recall in it many of the symptoms that Dr. Arkin described: a septic type of fever, hematuria, I think, pains in the limbs, and then symmetrical gangrene of the feet, and death. It was noticed that some of the arteries were diffusely thickened.

When I passed through London on my return several months later, Dr. Boycott had slides which showed a very distinct acute inflammatory process involving the various coats, somewhat resembling this, but without any nodosities. There, apparently, had been no ruptured hemorrhage, no healed aneurysm. It is probable at least that that also belonged to this type and that we must think of periarteritis nodosa as a disease that may be much more than a periarteritis and often not nodose.

Dr. Arkin is probably also aware of an infectious type of this disease that is supposed to occur in horses, which perhaps would be some answer to Dr. Wadsworth's query.

(Dr. Arkin, closing.) In answer to the question about hypertension, I want to say that in five cases I have seen, hypertension has been present only in those cases where very marked renal changes were shown. There may be a nephritis, or only extensive infarction, or both. In cases where there were no marked renal changes, there was, as a rule, no hypertension. In fact, the blood pressure was low.

In answer to the question about changes similar to these in animals, I want to emphasize that these changes were limited strictly to the arteries. There were no changes in the veins. The disease has been found in lower animals. It has been described in the calf, swine, dog, deer. It sometimes occurs in epidemic form. The histological changes resemble those in man.

I am glad Dr. LeCount called attention to the possibility of trauma as the case of periarteriolar hemorrhages. I simply showed it because it was found in the same organs where the other changes were present. Whether or not these are due to periarteritis nodosa, I can't state definitely. The brain was removed as carefully as possible.

The healed case is striking because the changes were all healed. In the cases of periarteritis nodosa that have been reported, inflammatory changes were also present. But to find a case such as this which was completely healed histologically, is a rare thing and I have not seen it before.

THE INVOLVEMENT OF MEDIUM-SIZED ARTERIES IN SYPHILITIC AORTITIS. Otto Saphir, Cleveland, O.

Abstract. Among 50 cases of syphilitic aortitis, the innominate artery showed syphilitic changes in 33, the carotid in 29, the superior mesenteric in 10, the inferior mesenteric in 3, the common iliac in 10, and the femoral artery in 7 cases. The subclavian artery was examined in 29 cases, 15 of which showed syphilis.

The syphilitic lesions were characterized by endarteritis of the vasa vasorum, and perivascular infiltration of lymphocytes in the adventitia. The media of the elastic type of arteries showed an interruption of the continuity of the elastic fibers and fibrotic areas combined with circumscribed lymphocytic infiltrations. The media of the muscular type of arteries only rarely showed changes.

In the intima, circumscribed button-like areas of fibrosis, without degenerative changes, were found very frequently. These are not specific but are chronic inflammatory in nature and might be associated with any type of pathological lesion in the adventitia.

Discussion

(Dr. B. J. Clawson, Minneapolis.) I should like to ask Dr. Saphir if he has had any experience with a coronary artery. I have examined coronary arteries in many cases of syphilitic aortitis and have not been able to find syphilitic lesions in them.

(Dr. A. Plaut, New York City.) What stain do you use? The orcein stain?

(Dr. Saphir, closing.) I have examined the coronary arteries in about 25 cases and I have never seen definite changes in the coronary artery directly attributable to syphilis. Spirochetes have never been seen in the aorta or in the vessels of the branches of the aorta.

The stains we use have been important. Warthin's stains and our stains, I think, have been instrumental in our success. The sections are placed in an alcoholic solution and then in the incubator for two hours; we then differentiate them with a 0.5 per cent solution of hydrochloric acid.

STUDIES ON THE PATHOGENESIS OF INFECTIOUS ENDOCARDITIS. K. Semsroth and Robert Koch (by invitation), Pittsburgh, Pa.

Abstract. 1. In paratyphoid or staphylococcus infected guinea pigs, hypersensitive to horse serum, there developed a nodular mononuclear endocardial proliferation with regularity, while non-sensitized controls only occasionally showed such reaction. Desensitization before infection practically prevents the reaction. Desensitization after the infection does not alter the result.

2. An allergic state was induced in rabbits by repeated intravenous *casein* injections. A hyperergic skin reaction was obtained after five to eight casein injections.

Subsequent infection with staphylococcus aureus led to a definite *endocarditis* in six out of eight animals.

ATTEMPTS TO REPRODUCE RHEUMATIC DISEASE IN ANIMALS. Louis Gross, Leo Loewe, and (by invitation) Benjamin Eliasoph, New York City.

Abstract. One hundred and two animals representing seven species were injected over a period of from one week to one year with twenty-one strains of streptococcus gamma, most of which were obtained from blood cultures of rheumatic disease cases. There was also injected a number of materials such as whole blood, serum, plasma, pericardial fluid, hydrocele fluid, filtrates of subcutaneous nodules, tonsils, lymph nodes, nasopharyngeal washings, etc. The majority of the material was obtained from cases where autopsy, biopsy (subcutaneous nodules), electrocardiograms, roentgenograms, and clinical findings made the diagnosis of rheumatic disease beyond question. Injections were made through a variety of routes, such as intracardially, intravenously, into the tonsils, nasopharyngeal mucous membrane, lungs, joints, peritoneal cavity, etc. In order to lower the resistance of the animals, a variety of procedures was

adopted, *viz.*, injections with Trypan blue, poisoning heart muscle with Digitan, subcutaneous injection of infected agar mass, thrombosis of superficial veins with subsequent infection of thrombus, etc. A number of lesions, including inflammatory myocardial foci, vegetative endocarditis, pericarditis and arthritis, were found. Criteria are discussed whose fulfillment are necessary in order to establish the experimental reproduction of rheumatic disease. According to these criteria, we have failed to reproduce the disease in animals.

Discussion

(Dr. B. J. Clawson, Minneapolis.) I should like to know what is meant by the typical Aschoff nodule.

(Dr. Gross.) Before I answer Dr. Clawson's question, I should like to take a moment or two in order to elaborate on several of the points which Dr. Loewe brought out.

I want to make it clear that we are not attempting to criticize caustically the past work in this field. Indeed, when we commenced our investigations several years ago, we were very hopeful of reproducing this disease by means of streptococci. However, in the light of our experience we must conclude that we have not reproduced rheumatic fever, nor do we believe that other workers have had any greater measure of success. On the other hand, we do not desire to leave the impression that we dogmatically deny the possibility that the streptococcus may be the etiological agent.

There are at present, broadly considered, three avenues of approach in the investigation of this disease: the bacteriological, the immunological and the anatomical. The bacteriological method has concerned itself with attempts to obtain organisms from rheumatic fever patients, principally from the blood, and to show by their relative frequency of occurrence, as well as by their serological identity, that they are responsible for the disease. Thus for example, Dr. Clawson found that seven of his ten streptococcus viridans strains fall into one serological group, and Dr. Kreidler found that 107 strains of his streptococcus and cardioarthritidis strains also fall into one group. I believe, however, that neither of these investigators carried out absorption tests to prove that they were not dealing with group agglutination. It seems that, as a whole, the bacteriological methods have led to no definite conclusions.

As examples of the immunological approach, one can briefly cite the work of Birkhaug on skin reactions, also the work of Dr. Swift and his collaborators. Dr. Birkhaug's work is very interesting, but it seems to me that all that we can conclude from his results is the fact that rheumatic fever patients show a marked skin susceptibility to certain streptococcus products. Dr. Swift and his co-workers have shown that the reactions in the tissues may depend on their immunological state. Attempts to interpret these interesting observations in terms of rheumatic fever, however, bring up many difficulties, not the least of which is the infrequent coincidental occurrence in the same heart of both subacute bacterial endocarditis and acute rheumatic disease, as evidenced by the presence of Aschoff bodies.

The therapeutic approach which properly falls under the immunological group presents obvious difficulties, for here one must eliminate non-specific protein effects and one must have clear-cut statistics on a large scale. An interesting example of the conflicting views presented by such work appears in the report of the Bath Conference on rheumatism, where Dr. Small presented his therapeutic results following the use of streptococcus cardioarthritidis serum

and vaccine, and Bertrand claimed even better results with his Achlame bacillus vaccine.

There is, therefore, left, as Dr. Loewe stated, the anatomical approach. He has shown you why our results lead us to the conclusion that the disease has not as yet been reproduced.

In answer to Dr. Clawson's question, I must say that, at any rate in our laboratory, we have the feeling that if there is any doubt in our minds as to whether a certain collection of cells is or is not an Aschoff body, we are not justified in calling it an Aschoff body. How much more critical, therefore, must we be in the case of experimental animals? Here the burden of the proof is obviously up to the experimenter.

(Dr. Clawson, closing.) I should like to correct Dr. Gross about my making claim to the specificity of the group of streptococci. I think if he refers to my paper he will find that I emphasized the fact that the streptococci isolated from patients with acute rheumatic fever did not represent a specific group. They were not a specific strain.

A Comparison of Rheumatic and Experimental Streptococcic Inflammations. Benjamin J. Clawson, Minneapolis, Minn.

Abstract. Rheumatic inflammation may exist as nodules or as irregular groups of large mononuclear and multinucleated cells, lymphocytes, plasma cells, eosinophils and a varying number of polymorphonuclear leucocytes.

The rheumatic nodule, called the Aschoff body when seen in the myocardium, is a small, fairly well localized area of inflammation, mostly polyblastic in character, but at times chiefly or entirely exudative. It varies greatly in size and shape. It may consist of but a few cells or be large enough to be seen with the naked eye. It may take the form of a rosette or become more or less fan-shaped or fusiform. The inflammation may extend out irregularly into the interstitial tissues and at times become so extensive as to form a diffuse myocarditis.

In a relatively high percentage of rabbits injected in the left ventricular cavity with agglutinated streptococci, nodular inflammatory areas, with a cellular reaction similar to that found in the human Aschoff bodies, may be produced in the myocardium.

The subcutaneous rheumatic nodule has a cellular reaction similar to that found in the Aschoff nodules in the heart. By injecting streptococci in small amounts into the subcutaneous tissues of rabbits, lesions can be produced which are morphologically similar to the nodules found in the subcutaneous tissues or the myocardium in cases of acute rheumatic fever.

The arterial lesions in cases of acute rheumatic fever consist of a swelling of the endothelium which becomes basophilic and may be raised from the basement membrane by a coagulated exudate. There is cellular necrosis in all parts of the vessel wall. Exterior to the necrotic wall there is a cellular exudate, many cells of which are polymorphonuclear leucocytes showing various stages of necrosis. In this area there are also plasma cells, and mononuclear and multinucleated cells similar to those found in rheumatic inflammation in other parts of the body.

Similar lesions may be produced in monkeys and rabbits by injecting streptococci intravenously and subcutaneously.

The similarity of the inflammatory reaction commonly found in cases of acute rheumatic fever to the reaction produced experimentally by injecting strepto-

cocci further suggests a causal relation between the streptococci and rheumatic fever.

Discussion

(Dr. K. Semsroth, Pittsburgh.) I should like to emphasize what Dr. Bell has said. While from a clinical and bacteriological standpoint it may be possible to differentiate between various forms of endocarditis, such a clear differentiation is *not* possible on the basis of the morphological picture of the heart lesions. "Rheumatic" myocardial nodules have been observed more than once in cases of non-rheumatic heart lesions. In our experience, intravenous treatment of rabbits with casein and subsequent staphylococcus infection was sometimes followed by the development of large mononuclear subepicardial nodules which looked much like several of the pictures which Dr. Clawson has shown.

(Dr. E. T. Bell, Minneapolis.) I should like to raise a question for those who think that rheumatic endocarditis and bacterial endocarditis are two distinct diseases. If you define rheumatic endocarditis anatomically as firm vegetations without thrombi and bacterial endocarditis as vegetations with thrombosis, you will find that half the cases of endocarditis are of mixed type, since both types of vegetations are present. Rheumatic vegetations are found in half the cases of clinical bacterial endocarditis. Are we going to argue that over half of the cases of rheumatic fever are merely complicated by streptococcus infection?

You will also find that the clinician will change his diagnosis after he watches the case a while. If the patient gets well it is rheumatic endocarditis. If he dies, it is bacterial endocarditis.

(Dr. L. Gross, New York City.) I should like to ask Dr. Clawson whether, if he were presented with slides from a human rheumatic heart and he did not know that the sections were from human material, he would under any circumstances be willing to commit himself as to whether the lesion is one of rheumatic fever, and, if so, what picture he has in mind when he so commits himself. Furthermore, how would he differentiate the Aschoff body from the type of multinucleated and large mononuclear collections frequently found in the rabbit's myocardium.

I should like to ask Dr. Clawson whether he has attempted to reproduce subcutaneous nodules by means of organisms other than streptococci.

Just one word about Dr. Bell's question. I was not quite clear as to whether Dr. Bell meant to indicate that he has found typical fresh rheumatic verrucae side by side with typical bacterial vegetations in so large a proportion of cases. I don't mean of course the typical healed verrucae which obviously represent previous rheumatism. I must say that our experience has been entirely different in this respect. We have found typical fresh rheumatic verrucae and Aschoff bodies coexistent with the typical subacute bacterial endocarditis, but this has been a very infrequent observation.

(Dr. Clawson, closing.) In regard to the question if a slide were placed before me from the heart of a human endocarditis as to whether I would select it as that of a human, I should say no. I do not think these nodules are specific for rheumatic infection. They are more common in rheumatic infection than in subacute bacterial endocarditis.

I am glad Dr. Gross brought that out because I was going to refer to the article describing a case of meningococcic endocarditis in which there were nodules which Dr. Rhoads and Dr. Mallory passed as Aschoff nodules.

Theodore Farr has long stressed the fact that he has found the Aschoff nodule in myocardium in cases of scarlet fever. Dr. Warthin has pointed out the simi-

larity between syphilitic infections and this type of infection. In going over the various cases of syphilitic aortitis in 8 per cent I found in the myocardium, near to the aortic ring, nodules which I would call Aschoff nodules.

In relation to the definition of Aschoff nodules, I would say: they are localized polyblastic areas of inflammation, but at times there is exudation to the extent of being almost an abscess, and I think we can find all stages from what is called the typical Aschoff nodule right down through to the abscess. Aschoff himself emphasized the fact that polymorphonuclear leucocytes were conspicuous in some of these nodules.

I want to emphasize the question of both lesions occurring in the same heart. We have had cases checked clinically where the clinician was not certain whether he had an acute rheumatic endocarditis or a bacterial endocarditis. Dr. Bell said it occurs in one-half of the cases. That is very conservative. I think in our paper we say three-fourths of the cases of subacute bacterial endocarditis show both types of lesions.

SKIN REACTIONS AMONG RHEUMATICS TO STREPTOCOCCAL PRODUCTS. Konrad E. Birkhaug (by invitation), Rochester, N. Y.

Abstract. During May to September, 1928, a series of 594 persons, among whom were 118 active and inactive cases of rheumatic fever, were studied for skin hypersensitiveness to streptococcal products. These cases were scattered in clinics in London and Birmingham (England), Bergen (Norway), Copenhagen (Denmark) and Hamburg (Germany). Altogether 3114 skin tests were made with one or several comparable concentrations of filtrates, autolysates and vaccines of hemolytic, viridans and indifferent streptococci, isolated from rheumatic fever sources. A marked universal hypersensitiveness was found to exist among active and inactive cases of rheumatic fever, carditis, or chorea, to filtrates, autolysates and vaccines produced by the viridans and indifferent streptococci, and moderately to the autolysates of the hemolytic streptococcus. Inactive cases of rheumatic infection react less to the non-hemolytic streptococcal products than active cases of rheumatic fever, carditis, or chorea. Filtrate boiled for four hours produces exaggerated skin lesions in active cases of rheumatic fever almost as frequently as the unheated filtrate, while reactions with the boiled filtrate are almost nil in inactive rheumatic infection. Salicylates exert no effect upon the capacity of skin to react hyperergically with the streptococcal products in active or inactive rheumatic infection. More than 70 per cent of rheumatic fever, carditis or chorea react positively to the non-hemolytic streptococcal products, while about 25 per cent of non-rheumatics give positive reactions. Cases of chronic infectious arthritides react to the viridans and indifferent streptococcal products in almost the same proportion as inactive cases of acute rheumatic infection, while degenerative chronic types of arthritides behave like non-rheumatic controls. It appears that a common allergenic factor is present excessively in the viridans and indifferent streptococcic cells and moderately in the hemolytic streptococcus, which reacts universally when injected intradermally in persons stigmatized by rheumatic fever. No evidence is available of a strictly specific allergenic agent. No parallel reactions occur in rheumatic infections with the Dick or Schick tests.

Discussion

(Dr. R. R. Mellon, Pittsburgh.) I should like to ask Dr. Birkhaug whether any of these patients have been tested out with organisms other than strepto-

cocci. I ask this question for two reasons: First, because I have had occasion from time to time to observe positive skin tests of rheumatics with certain other organisms to which they appeared to be abnormally sensitive, and partly because we were impressed with this non-specific or allergic factor in my work with Drs. Koch and Semsroth, particularly as evidenced by the non-specific action of casein in our experimental cardiac localizations.

(Dr. Birkhaug, closing.) It was desirable to perform a great many more skin tests on these children and adults with heterogenous bacterial substances. But to perform nine skin tests, particularly on a child, is a pretty gruesome ordeal, especially when done in the out-patient clinic. That is the sole reason for confining these studies to the three varieties of streptococci. Further studies, however, are contemplated wherein products of other organisms will be employed.

DOES SPLENECTOMY INFLUENCE THE LEUCOPENIA INDUCED BY CERTAIN FOREIGN SUBSTANCES? Isolde T. Zeckwer, Philadelphia, Pa.

Abstract. Doan, Zerfas, Warren, and Ames (*J. Exper. Med.*, 1928, xlvii, 403) have recently reported interesting experiments in which they found that sodium nucleinate injected intravenously in normal rabbits resulted in a marked leucopenia in the peripheral blood, which lasted for a number of hours, when it was succeeded by a leucocytosis. They believe that during the peripheral leucopenia, the leucocytes have collected exclusively in the spleen, as indicated by leucocyte counts made on the blood from the viscera, and by their finding that after splenectomy, sodium nucleinate resulted in no period of leucopenia, and that leucocytosis then began within a period of time one-half to one-sixth of that required in animals in which the spleen was intact.

It seemed of importance to determine whether these findings of the effect of splenectomy are of universal application to the reaction to foreign substances in general or apply only to the reaction to the specific substance sodium nucleinate. If the spleen has such a function of storing and then discharging leucocytes, it would be of as great significance as the mobilization of red cells by the spleen from its reserve, in conditions of great need, as demonstrated by Barcroft.

To determine this point, *B. coli* vaccine was injected intravenously into rabbits, and leucocyte counts were made on the ear blood at frequent intervals after injection. The leucocytes fell abruptly and remained at a low level for several hours. The curves thus obtained were compared with curves obtained after vaccine in the same animals at different times after splenectomy. It was found that splenectomy had no effect on the degree of leucopenia, or on the time of rise of leucocytes after the leucopenic period.

These results necessitated a reinvestigation of the effects of splenectomy on the leucocyte changes following the injection of sodium nucleinate. Doan and his associates used sodium nucleinate in doses of 1 gm. per rabbit. When this dosage was used in the present experiments, the animals showed marked circulatory depression. It was found that a much smaller dose, 0.1 gm. per rabbit, was just as effective in producing leucopenia and resulted in no obvious vascular changes, so that the animals could be bled readily, and this dosage was well tolerated, so that injections could be repeated frequently for comparison. It was found that in every animal used, splenectomy did not prevent the occurrence of a marked leucopenia, in doses of 0.1, 0.5, and 1 gm. The time of rise in leucocytes varied greatly on different occasions in the same animal before splenectomy, and there was no evidence that splenectomy resulted in a more

rapid return rise of the leucocytes than the same animals had shown before splenectomy.

Blood pressure tracings were made in several animals. With 1 gm. doses the blood pressure fell to a low level on injection of sodium nucleinate, and this low level was maintained for a long time. With 0.1 gm. doses there was a transient fall in blood pressure, but a rapid return to normal, and the leucopenia was maintained while the blood pressure remained at the normal level.

It is concluded that the leucopenia following injections of sodium nucleinate, or bacterial vaccines, is not of splenic origin, and that there is no evidence in these experiments that the spleen exerts any regulatory function over the distribution of leucocytes within the body.

EFFECT OF REPEATED EXPOSURE TO HIGH TEMPERATURE ON THE LEUCOCYTE COUNT IN MICE. O. R. Caillet (by invitation) and J. P. Simonds, Chicago, Ill.

Abstract. Murphy and Sturm showed that a definite leucocyte curve could be produced by subjecting mice, on a single occasion, to dry heat. Nakahara, in the same series of animals, demonstrated changes in the lymphoid tissue which he considered definitely associated with the blood picture produced by the dry heating. It has been the object of this study to observe the effect upon the blood and lymphoid tissue of repeatedly submitting mice to dry heat in the manner described by the workers cited above. Groups of mice were heated for five minutes at a temperature of 60° C at intervals of ten days over a period of two hundred and ten days. During the first fifty days there was a marked fall in the white cell count after each heating, accompanied by cellular necrosis in the spleen and followed by the appearance of many mitotic figures in the germinal centers, and a rapid rise in the white cells in the blood stream. During this initial period there was a stair-step rise in the white cells principally lymphocytes up to 25,000 per cmm. During the remainder of the period of heating there was a gradual fall to and below the normal leucocyte count. The fluctuation following each heating was very small. Sections of the spleen at this time showed a marked fibrosis with loss of germinal centers.

Discussion

(Dr. W. J. MacNeal, New York City.) The paper is before you for discussion.

I should like to ask whether there were any observations upon the internal temperature of the mice immediately following the exposure to heat.

(Dr. Caillet.) In our observations we did not notice the internal temperature. However, Murphy noticed an internal temperature rise of about one to two or three degrees centigrade immediately following the heating.

(Dr. MacNeal.) Maximum about 40° to 42°?

(Dr. Caillet, closing.) Not so much, I should say.

COAGULABILITY OF THE BLOOD DURING MECHANICAL OBSTRUCTION OF THE HEPATIC VEINS. W. W. Brandes (by invitation) and J. P. Simonds, Chicago, Ill.

Abstract. The changes that occur after constriction of the hepatic veins by a method previously described by us are: (1) a precipitate fall in arterial blood pressure; (2) a decrease followed by a slower increase in blood concentration; (3) a decrease in sugar content of the blood; and (4) a decrease in coagulation

time. After release of the constriction of the hepatic veins the blood pressure rises sharply, a temporary hyperglycemia is produced and the coagulation time is increased. During the constriction the liver shows marked congestion, and pressure in the portal vein is increased.

Various studies have been attempted in an endeavor to explain the decrease in coagulation time, but as yet no definite conclusions have been reached. Since a normal dog may show variations in coagulation time, it is difficult to interpret the results obtained. That this decrease in coagulation time after constriction is not due to the anesthetic and trauma is, we believe, shown by the fact that it occurred also in dogs which had been kept under ether anesthesia and subjected to trauma for varying periods of time before the hepatic veins were constricted. The average coagulation time in fifteen dogs before constriction was three minutes, while within five minutes after constricting the hepatic veins it was one minute fifteen seconds.

Changes in platelet counts are not sufficiently marked to account for the change in coagulation. There seems to be a general tendency to a slight decrease throughout the experiment, but there is no such decrease as occurs in peptone shock.

Fibrinogen determinations were not constant. But there appears to be a slight tendency to a decrease in fibrinogen during constriction of the hepatic veins.

Blood calcium shows but little variation during the period of constriction. In these experiments the hydrogen ion content of the plasma showed a moderate increase.

From the above it is difficult to explain the cause of the change in blood coagulability. Shutting the liver out of the circulation may prevent heparin from entering the circulation. Also in course of the changes in the concentration of the circulating blood, constituents which hasten coagulation may enter the circulation with the tissue fluids. After hemorrhage we know that the coagulability of the blood increases. Furthermore dilution of the blood may be a factor in this decrease of coagulation time.

Discussion

(Dr. Simonds.) There is one thing that might be added to what Dr. Brandes has said. It is very well known that in peptone shock there is a marked reduction in number, and sometimes an almost complete disappearance, of blood platelets from the peripheral blood. A considerable number of counts of blood platelets during constriction of the hepatic veins showed that the variations were entirely within the possibility of error by the method used.

THE EFFECT OF UNILATERAL NEPHRECTOMY ON THE TOTAL NUMBER OF FUNCTIONING GLOMERULI IN THE RABBIT. Robert A. Moore and Gregory F. Lukianoff (by invitation), Cleveland, O.

Abstract. Using the method of Hayman and Starr, the total number of open glomeruli in the kidney of the rabbit has been determined by an intravital injection of Janus Green B. The normal number of open glomeruli was found to vary from 44 to 75 per cent, with an average of 63 per cent. For one to ten days following unilateral nephrectomy, the total number of open glomeruli was found to vary from 90 to 100 per cent. Control animals subjected to a sham operation failed to show any variation from the normal. We believe that the immediate compensation for the loss of one kidney is accomplished by this increase in the number of open glomeruli.

Discussion

(Dr. E. T. Bell, Minneapolis.) I should like to ask Dr. Moore what percentage of the total capillary circulation is in use in a normal resting kidney. In his estimation he showed about 63 per cent on the average of the glomeruli in action but only parts of many glomeruli were open.

(Dr. Moore, closing.) To answer Dr. Bell's question would involve the question of the intraglomerular circulation. I have no quantitative studies, but it is my impression that approximately one-third of the capillary beds of the 63 per cent of open glomeruli are in circulation. The other two-thirds are out if we judge by this method of staining.

THE PRODUCTION OF ALLERGIC INFLAMMATION IN THE KIDNEYS. Opal E. Hepler (by invitation) and J. P. Simonds, Chicago, Ill.

Abstract. A local reaction similar to the Arthus phenomenon has been produced in the mucosa of the stomach (Schapiro and Ivy) and in the lungs (Friedberger and Opie) of animals previously sensitized to the protein injected. We have studied the local reaction in the kidneys of sensitized rabbits following the injection of egg albumin and horse serum directly into the left kidney. From one to eight intrarenal injections were given under aseptic precautions at five-day intervals, with and without a preliminary series of subcutaneous injections. The animals were killed from twenty-four hours to one month after the last injection. It was found that the injection usually produced local hemorrhage. When the antigen was injected into the cortex of the kidney, necrosis involving especially the tubular epithelium, but also, to a less extent, the glomeruli and connective tissue, occurred. This was accompanied by more or less edema and leucocytic infiltration of the area of necrosis. Inasmuch as these changes did not follow a single injection of foreign protein into the kidney of a non-sensitized animal, we believe that the changes described were due to the action of the antigen upon the locally sensitized renal tissues.

Discussion

(Dr. E. R. Long, Chicago.) This is of great interest to me in connection with results that I reported before this Society last year. I found that in tuberculous animals, when the specific product to which they were sensitive (tuberculin protein) was introduced in the kidney, distinct changes took place. Those were of more definitely inflammatory character than these which have just been reported. In only one instance was there a change which was rather similar to those just described. This was in an animal previously normal which was simply injected with a tuberculin protein. On reinjection with the same material two weeks later, a considerable amount of necrosis occurred, which I interpreted as an Arthus' phenomenon. The necrosis seemed to me to be all out of proportion to any vascular damage that could be seen in the sections. This, I think, is quite in harmony with what you have reported. Yesterday, Dr. Lewis and Dr. Seibert reported experiments on this same protein, indicating that it elicits antibody production.

(Dr. E. Ecker, Cleveland.) I should like to ask Dr. Hepler what reactions occurred when she introduced the needle alone into a kidney.

(Dr. Hepler, closing.) We injected four rabbits in all and checked them immediately, one-half hour, one hour and two hours after we injected them. All we observed was a hemorrhage, no necrosis.

THE FINER STRUCTURAL CHANGES IN THE GLOMERULI IN GLOMERULONEPHRITIS. Leone McGregor (by invitation), Minneapolis, Minn.

Abstract. A histological study of the changes in the glomeruli in glomerulonephritis has been made using Mallory's aniline blue (Heidenhain azo-carmine modification). This stain brings out the glomerular basement membrane distinctly, thus separating the endothelial from the epithelial cells.

The changes observed in the earliest stages of glomerulonephritis were as follows: (1) a marked accumulation of mononuclear and a few polymorphonuclear leucocytes within the capillary loops; (2) the appearance of delicate blue-staining hyaline fibers between the leucocytes; (3) a slight increase in endothelial cells; (4) a proliferation and often a degeneration of the glomerular epithelium; (5) a proliferation of the capsular epithelium with crescent formation; (6) the presence of hyaline blue-staining fibers between these capsular epithelial cells.

In later stages of the disease the intracapillary hyaline fibers form a definite meshwork enclosing the leucocytes and a few endothelial cells. The meshwork becomes more dense until the lumen of the capillary is almost filled with a mass of hyalin containing some cells. The blood circulation continues between the basement membrane of the loop and the periphery of the hyaline mass. Finally the mass completely occludes the capillary. Adjacent loops fuse with one another and with the capsule to produce a hyaline glomerulus.

This process is typical of all cases which are clinically glomerulonephritis.

Preliminary studies on hypertension kidneys show a different glomerular lesion consisting of an irregular thickening of the basement membrane. Intracapillary hyaline fiber formation is seen only in those hypertensives who have died of uremia and then in only a few glomeruli.

Examination of amyloid kidneys has confirmed Ohmori's observation that the amyloid is deposited inside the glomerular basement membrane.

Discussion

(Dr. E. T. Bell, Minneapolis.) I would like to say that these lantern slides do not show as well as the drawings. The lantern slides were made from colored drawings, and the membrane does not come out as well as it should. This is really an excellent stain for studying the finer changes in glomeruli.

The basement membrane is brought out sharply and with the aniline blue stain you are able to tell what is inside and what is outside of the capillary.

The hyaline fibers which Dr. McGregor has not been able to identify chemically seem to be typical of glomerulonephritis.

LIPOID NEPHROSIS. E. T. Bell, Minneapolis, Minn.

Abstract. A survey of recent literature shows a tendency to use "lipoid nephrosis" in a broad way to include all cases of nephritis with edema, albuminuria, normal blood pressure and normal blood nitrogen. This clinical definition includes many cases that are anatomically acute or mild chronic glomerulonephritis.

All the clinical phenomena of lipoid nephrosis, *viz.*, albuminuria, edema, normal blood pressure, hypercholesterolemia, decrease of the plasma proteins with reversal of the albumin-globulin ratio and normal blood nitrogen, may occur usually in a less pronounced form in glomerulonephritis.

There are many transition cases between lipoid nephrosis and glomerulonephritis, *viz.*, those that fit the definition of lipoid nephrosis except for the pres-

ence of hypertension or moderate nitrogen retention. These are called the mixed type or nephritis with a nephrotic *Einschlag*. From the clinical standpoint the two diseases cannot be sharply separated.

A histological study has been made of ten cases of large, fatty kidneys, without tubular atrophy, that were clinically nephritis. Four of these are classed as pure lipoid nephrosis, three as nephritis with nephrotic *Einschlag* (mixed type) and three as of uncertain type because of lack of clinical data.

The glomeruli are not normal in any instance, but the lesions are not of uniform type.

In pure lipoid nephrosis there is an uneven thickening of the basement membrane of the glomerular capillaries, and there may be many mononuclear leucocytes in some of them.

In the mixed type (nephritis with nephrotic *Einschlag*) there is a marked thickening of the basement membrane. In one instance the capillaries were compressed by enlarged epithelial cells. In two instances there were many leucocytes in the capillaries, and a few areas in the glomeruli showed the changes characteristic of typical clinical glomerulonephritis, *i. e.*, capillaries filled with leucocytes and a network of hyaline fibers.

Three large non-contracted kidneys were studied in which the data were insufficient to determine whether they should be classed as lipoid nephrosis. Two of these showed extreme thickening of the basement membrane. The third showed an enormous accumulation of fat in the tubules and glomeruli, large glomerular epithelial cells with hyaline and fatty degeneration, and leucocytes and hyaline fibers within the capillaries as in glomerulonephritis.

Lipoid nephrosis is to be regarded as a form of glomerulonephritis in which the glomeruli are damaged but their capillaries are only partially obstructed so that they continue to function and tubular atrophy does not occur.

Discussion

(Dr. E. R. Long, Chicago.) It is quite astonishing what changes can be brought out by the staining methods of Drs. McGregor and Bell. Many of those glomeruli appear to be normal until subjected to the special new stain. I should like to ask if they have any idea of the age of those lesions characterized by the thickening of the basement membrane shown by this technique.

(Dr. W. J. MacNeal, New York City.) I should like to ask Dr. Bell whether in his cases of lipoid nephrosis he has examined the other organs, as, for example the liver, for lipoid-containing cells; whether perhaps we may not be dealing with a much more widespread process than that merely involving the kidney.

(Dr. H. G. Wells, Chicago.) I should like to add for a little expression of satisfaction that I have been looking all my life for pure, chronic lipoid nephrosis in the adult. At autopsy I have had a number of cases in which the clinical picture was absolutely classical and the gross picture was absolutely classical of chronic lipoid nephrosis, and then, to my horror, I found typical glomerular changes of subacute or chronic glomerulonephritis.

(Dr. Bell, closing.) I do not know the significance of the hypercholesterolemia. We have no knowledge concerning that at all.

I think lipoid nephrosis is a renal disease because it shows many transitions to glomerulonephritis. Hypercholesterolemia is often pronounced in typical cases of glomerulonephritis.

There is a widespread opinion that lipoid nephrosis is a metabolic disorder,

but all the symptoms and laboratory findings characteristic of lipoid nephrosis may be found in glomerulonephritis, which is surely a renal disease.

In reply to Dr. Long's question, the case that showed the most striking hyaline changes in the basement membrane was of fifteen years duration. The patient had had edema for fifteen years. There never was renal insufficiency nor cardiac hypertrophy.

We have no accurate information concerning the duration of the other cases. The best case we had was of four weeks duration.

I have not studied the changes in the other organs as extensively as I should in order to reply fully to Dr. MacNeal. In the cases which I have studied I did not see any fatty changes in the spleen or in the liver, and there is no similarity to Niemann's disease.

CALCIFICATION OF THE KIDNEYS IN BISMUTH POISONING. C. V. Weller, Ann Arbor, Mich.

Abstract. In bismuth poisoning in man, calcification of the damaged renal tubular epithelium is found, comparable to that described for experimental bismuth nephritis in lower animals. The chief deposits of lime salts are in the straight tubules of the medullary rays and in that portion of the ascending limb of Henle which returns to pass near the glomerulus of origin. While bearing a general resemblance to the lesions produced by mercuric salts, there are minor differences which, if they are found to be constant upon further observation, may assist in differentiating the two conditions.

PATHOLOGICAL EFFECTS FOLLOWING EXCESS OF IRRADIATED ERGOSTEROL. Harry Goldblatt, and (by invitation) A. T. Shohl, Cleveland, O.

(*Abstract not received.*)

MULTINUCLEATE CELLS IN THE SEMINIFEROUS TUBULES OF THE TESTIS. David P. Seecof, Denver, Colo.

Abstract. Multinucleated or so-called "giant" cells were present in practically every seminiferous tubule of the testes of a man 42 years of age dying of septicemia three days after the onset of an acute sore throat. The "giant" cells were formed chiefly from spermatids by a process of fusion. A few similar "giant" cells and others resulting from a fusion of spermatocytes and spermatogonia were found in the testes of some individuals of all ages dying of various acute and chronic diseases. A specific etiological factor for the lesion could not be determined. The findings were in accord with those of previous writers on the subject as regards the occurrence and pathogenesis of this lesion, and a brief summary of the literature is given.

CYSTITIS EMPHYSEMATOSA; REPORT OF CASES IN MEN. Ralph G. Mills, Rochester, Minn.

Abstract. Cystitis emphysematosa, judging from the literature, is a rare condition, having been reported only eleven times by six observers. A similar condition has been seen in animals, and in these also has been confined to the female sex. The discussions have been intermingled with reports of *colpohyperplasia cystica*, and with *pneumatoides cystica intestinorum* in a very confusing manner. Studies involving the histology as well as bacteriology and chemical

examination of the gas content of the vesicles have been made on all three conditions and applied indiscriminately to the others. Opinions have varied as to whether cystitis emphysematosa is a postmortem invasion by some gas-forming organism, or whether it really exists during life. There are no reports indicating *intra vitam* diagnosis.

This study is part of a series of articles discussing various phases of the subject, and attempting to establish this condition on an independent basis, free from inference or comparison with the others mentioned. It disposes of the presumed sex-selective nature of the lesion, and tends to show that it develops during life. It indicates that a real cystitis is a part of the process, and that the vesicles are not cysts in the strict sense, being devoid of a true lining of epithelial or endothelial cells, or their derivatives. The designation "cystitis emphysematosa" apparently conveys the correct interpretation.

Discussion

(Dr. Hueper, Chicago.) I reported one case several years ago; I think that the etiology of this condition can be clarified only by a thorough analysis of all cases observed. Unfortunately, this is not always possible and was not done in my own case as I received the tissue from the physiological department of Loyola University. I attempted to reproduce this condition in male rabbits by blocking the perivesicular lymphatics by injection of liquefied paraffin, followed by a caustic destruction of the bladder mucosa with a concentrated silver nitrate solution and subsequent injection of a bouillon culture of various strains of *B. coli*. The rabbits received moreover intravenous injections of a 10 per cent glucose solution. But the result was negative.

Some Swedish authors could reproduce the intestinal emphysematosis by the injection of gram negative bacteria cultured from the lesions of human material. I believe that the cystitis emphysematosa is also due to a bacterial infection and that cultures made from the lesion will help to establish the etiology.

INTRANUCLEAR INCLUSIONS IN YELLOW FEVER. E. V. Cowdry and S. F. Kitchen (by invitation), St. Louis, Mo.

Abstract. Intranuclear inclusions like those described by Torres in the liver cells of monkeys infected with the Brazilian virus of yellow fever were likewise found in fifteen monkeys inoculated with the African virus of the disease. They were also observed, though less abundantly, in human cases both from the Americas and from Africa. Moreover, the character of the intranuclear inclusions is such as to lead us to classify the virus of yellow fever with other filterable viruses which produce distinctive nuclear inclusions.

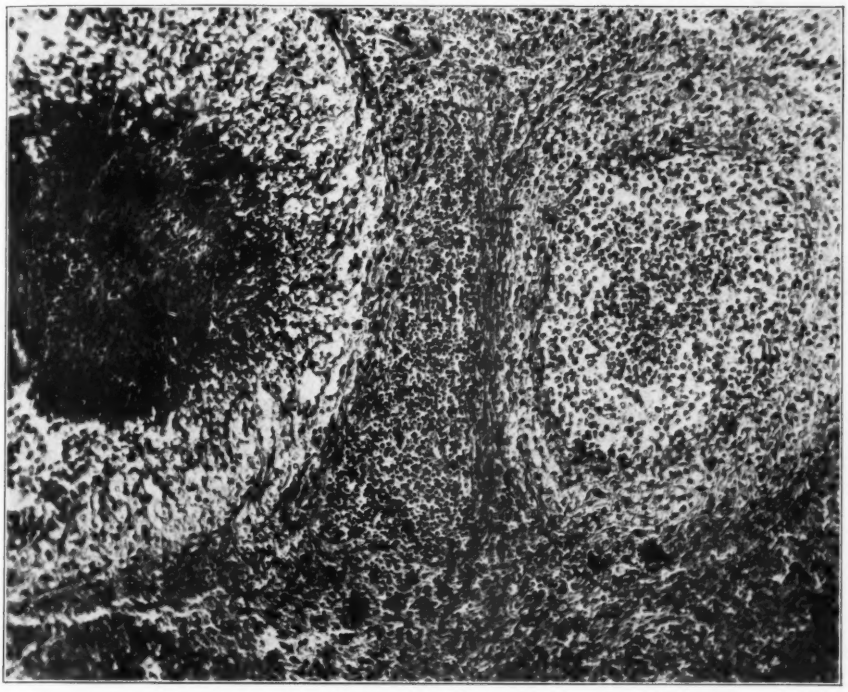
Discussion

(Dr. E. B. Krumbhaar, Philadelphia.) Dr. Cowdry's paper is open for discussion.

I should like to ask Dr. Cowdry if he would venture any statement as to his ideas on the production of these inclusions in yellow fever and in this group of conditions.

(Dr. Cowdry, closing.) I do not wish to commit myself, but we have no reason to suppose that the inclusions are anything other than a degeneration, what is called an oxychromatic degeneration. We have looked for materials in the

nucleus which should not be there, which might be conceived to come from without, but we have not found them. We have tested the microchemistry of the inclusions as far as we can go, and have found that they do not contain any detectable amount of massed iron as revealed by the MacCallum–Bensley method. Neither do they contain thymonucleic acid. I think I have nothing further to add. It is just a case of a peculiar change in the nucleus which we have associated with cases of yellow fever from these various sources and which we have not been able to observe in lesions in any other disease.



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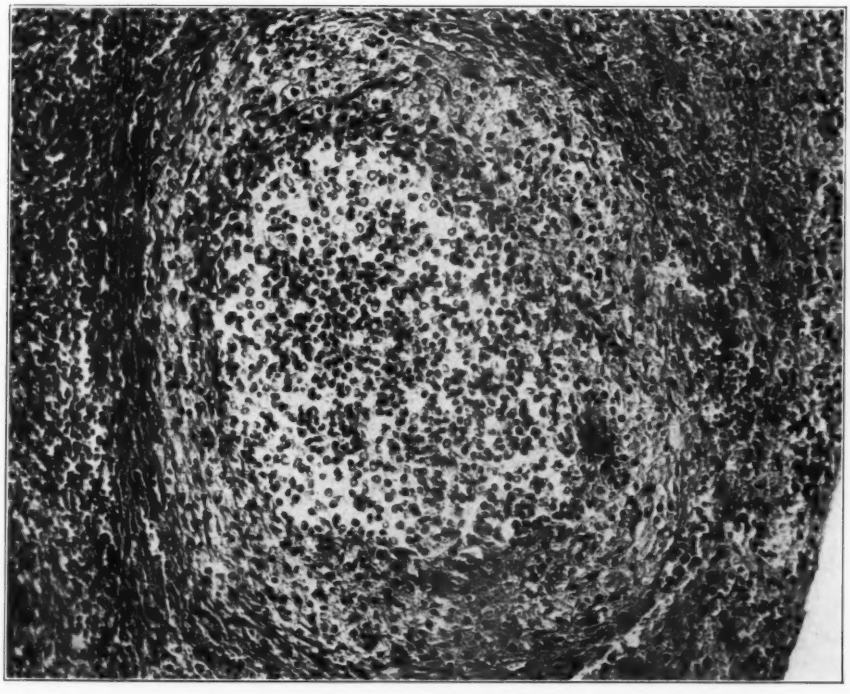

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Feldman Multiple Focal Splenitis of Guinea Pigs